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Appendix

Section I

1. Physiological Study of Graft Union Formation in Cactus II. Role of Auxin on Vascular Connection between Stock and Scion. Takashi SHIMOMURA and Kenzo FUJIHARA (Lab. Floricul.) J. Jap. Soc. Hort. Sci. (Japan). **45**, 397–406 (1977)

Young seedlings of *Notocactus submammulosus* var. *pampeanus* cut transversely were placed onto transverse cut surfaces of young plants of *Hylocereus trigonus*. Vascular connection between stock and scion occurred in almost all grafts 20 days after grafting when cut ends of vascular bundles of stock and scion were placed together (Method I). When they were 1 or 3 mm apart transversely to each other (Method II or III), vascular connection occurred in only 40 or 10% grafts.

By the Method II, NAA, IAA and 2, 4–D promoted vascular connection, and also increased the diameter of the vascular bundle connecting those of stock and scion. NAA (100 ppm) was most effective when applied three times every three days after grafting.

TIBA (0.1% in lanolin), a well-known inhibitor of basipetal transport of auxin, inhibited vascular connection strongly. But, by subsequent NAA (0.01% in lanolin) applications, this inhibitory effect of TIBA was effaced completely.

When lanolin with NAA (0.01%) was applied instead of a scion, it also induced a cambium in the area of the tissue between the lanolin and the vascular cut end of the stock.

The results suggested that the vascular connection in cactus grafts may be controlled by endogenous auxin of scions.

2. Prevention of Auxin–induced Vascular Differentiation in Wound Callus by Surface-to-Surface Adhesion between Calluses of Stock and Scion in Cactus Grafts. Takashi SHIMOMURA and Kenzo FUJIHARA (Lab. Floricul.) Plant & Cell Physiology (Japan). **19**, 877–886 (1978)

The relationship between formation of CVB, which connects vascular cut ends of stock and scion, and the condition of tissue where CVB is expected to be induced was studied using a graft combination of *Notocactus submammulosus* var. *pampeanus* young seedlings on *Hylocereus trigonus* plants.

When a scion with its basal part removed by a transverse cut was put onto a transverse cut surface of a stock with a distance of 1 mm between the vascular cut ends of both graft components, CVB was formed in only 13% of the grafts in the callus mass made by adhesion between calluses of stock and scion. The percentage arose to ca. 100% when 100 ppm NAA was applied to the scion apices. This promotive effect of NAA decreased with increasing time from grafting to NAA application.

When wound calluses of stock and scion were prevented from contact only at the portion between the vascular cut ends of stock and scion by introducing a piece of aluminium foil into the graft union, CVB was formed in 80% of the grafts in the callus of the stock without NAA application. In the case where the scion was grafted at right angle onto an additional longitudinal cut surface made 1 mm from the vascular bundle of the stock with remaining intact parenchyma between this cut surface and the vascular bundle of the stock, CVB was not formed even when NAA was applied. These results suggested that wound response was a prerequisite for CVB differentiation and that surface-to-surface adhesion of calluses inhibited CVB formation by making callus cells differentiate into quiescent parenchyma cells rapidly. The mechanism of this phenomenon is discussed.

3. Regeneration of Bulblets of *Hippeastrum* Bulb Segments Excised from Various Parts of a Parent Bulb. Tadashi YANAGAWA and Yoshihiro SAKANISHI (Lab. Floriculture) J. Japan Soc. Hort. Sci. (Japan). **46**, 250–260 (1977) (*in Japanese*)

Studies of *in vitro* culture were carried out to make a comparison of the responses in adventitious bulblet formation between tissues excised from different parts of parent bulbs. The culture medium used was White's supplemented with 2% sucrose and 0.8% agar. No exogenous growth regulators were supplemented. All cultures were maintained at 25°C under dark. A preliminary test showed that 25°C and 30°C were best for the culture, and that illumination was not necessarily required for bulblet formation itself, though it hastened the growth of the bulblets formed. The test also demonstrated that the cultures showed no seasonal response with respect to regeneration.

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1. *Longitudinal position on a scale.* Segments containing both tissues of scale base and basal plate produced bulblets from the proximal end of the scale and roots from the basal plate tissue at a high rate. Segments of the extreme base of scale also produced bulblets and the bulblets rooted later. In these cases, a higher regenerative rate was found for outer scale segments than for inner ones. Segments whose proximal end had been located 2 mm above the junction of the basal plate produced bulblets at the rate of only 3% and no regeneration was found from those 3 mm and more above the junction. Explants of bulb plate tissue alone regenerated neither roots nor bulblets.

2. *Position around a scale base.* For all circular scales (leaf bases), the leaf-blade-extended-side is thicker than the opposite side. As successive leaf blades extend 180° apart around a bulb, the thicker part of a scale and the thinner part of the next scale are arranged alternately. In the culture of segments of a single scale base, either with or without basal plate tissue, no difference in regenerative rate was shown between those excised from the thicker and thinner parts of a scale. In segments, each containing two thin and thick scale base fragments attached to basal plate tissue, bulblets developed at a higher rate and more rapidly from the position between the scale fragments than from either of the scale surface exposed sides. In a comparision of the two scale fragments with respect to regeneration from the surface exposed sides, the thinner one showed a clear tendency to produce bulblets at a higher rate than the thicker one. Lower regenerative ability of the thicker part, which was not clarified by the culture of a single fragment, appeared to be due to competition of the two segments.

3. *Dorsiventral polarity of regeneration.* Although some explants with basal plate tissue planted with the adaxial surface upwards regenerated bulblets from the adaxial side of the scale base, most explants regenerated bulblets from basal abaxial areas alone, regardless of the orientation of planting. When segments of a scale base with basal plate tissue were bisected parallel to their two surfaces and cultured, more than 60% of the adaxial halves which were planted erectly or with the adaxial surface upwards regenerated bulblets at their proximal end. When segments having both surfaces were cultured in a liquid medium by a reciprocating and a rotary shaker, 17% and 10% of the segments, respectively, produced bulblets on the adaxial side. These results suggest that some substance involved in the regenerative response and transmittable across the tissue may be more richly concentrated in the abaxial side than the adaxial side of the scale base.

4. Factors Affecting the Growth of *in vitro* Cultured Lateral Buds from *Phalaenopsis* Flower Stalks. Michio TANAKA and Yoshihiro SAKANISHI (Lab. Floriculture) Scientia Hortic. 8, 169–178 (1978)

Phalaenopsis flower-stalk buds cultured in vitro show 3 modes of growth: dormant, vegetative and reproductive. The effects of bud position on the stalk, temperature, and benzyladenine (BA) on the mode of bud growth were examined.

Flower stalks were cut into 1-node sections, each with 1 bud, and inserted into solid culture midium, top side up. Buds on the upper sections had a tendency to remain dormant regardless of temperature. Sprouting buds placed at 20°C or 25°C showed reproductive growth except for some on the basal sections which grew vegetatively. At 28°C, all buds developed vegetatively independent of their original position on the stalk. The buds on the sections taken from both stalks which had elongated at min 18°C and min 28°C were subcultured; the mode of growth of the cultured buds was not affected by the temperature during flower-stalk elongation but was affected by the subsequent culture temperature.

Cultured buds which had remained dormant were stimulated to sprout by addition of BA to the medium. At 5 p.p.m. and above, all buds began to develop, but malformations developed on the leaves of the shoots. All factors considered, BA at 2.5 p.p.m. was found to be the optimum level. The further development of the buds released from dormancy by BA was also affected by the position on the flower stalk and by cultural temperature.

5. Chilling Injury in Eggplant Fruits. IV. Ultrastructural Changes of Cell and Organelle Membranes Associated with Chilling Injury of Eggplant Fruits. Kazuhiro ABE and Kuniyasu OGATA (Lab. processing and physiology of horticultural products) J. Japan. Soc. Hort. Sci. (Japan), 46, 541–547. (1978) (*in Japanese*)

This paper reports the ultrastructural changes associated with chilling injury of eggplant fruits based on the electron microscope.

1. By using an electron microscope it was observed that cytoplasm in epidermal cells of eggplant fruit had little difference from other fruits and vegetables in general. Parenchyma cells had less cytoplasm than epidermal cells beside the cell wall and the ultrastrucures of cytoplasm were the

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same as those of the cytoplasm in epidermal cells.

2. The ultrastructural changes and cell wall burst of epidermal and parenchyma cells did not occur after 9 days' storage at 20°C. Eggplant fruit showing no pitting injury after 4 days' storage at 1°C showed ultrastructural changes of cytoplasm in parenchyma cells such as partial degradation of tonoplast and swelling of mitochondria. The epidermal cells of the pitting portion in eggplant fruit after 9 days' storage at 1°C displayed no cell wall burst but nuclear membrane, mitochondria, and ground substance had ultrastructural changes. The parenchyma cells of the pitting portion in eggplant fruit after 9 days' storage at 1°C showed cell collapse without cell wall burst and ultrastructural changes such as degradation of tonoplast and swelling of mitochondria having irregular distribution of cristae in them. In the parenchyma cells near the pitting portion, no cell wall burst occured but tonoplast, mitochondria, and ground substance had slight changes which bore resemblances to the changes in the pitting portion.

3. In the parenchyma cells of the pitting portion, globules which showed parallelogram cut were observed in vacuole sap. Each globule usually contained a cross shape which was relatively highelectron dense.

6. Chilling Injury in Eggplant Fruits. V. Changes of K Ion Leakage and Contents of Phopholipids during Storage and Effects of Phenolic Compounds on K Ion Leakage, Phospholipid Content and Ultrastructural Changes of Eggplant Fruit Sections. Kazuhiro ABE and Kuniyasu OGATA (Lab. processing and physiology of horticultural products) J. Japan. Soc. Hort. Sci. (Japan) **47**, 111–120. (1978) (*in Japanese*)

This paper reports the changes of K ion leakage from sliced tissues and contents of phospholipid in eggplant fruits during storage. Effects of addition of phenolic compounds on K ion leakage, phospholipid contents, and ultrastructural changes of eggplant fruit sections were studied.

K ion leakage of eggplant fruits stored at 20°C did not change during storage. In contrast K ion leakage of eggplant fruits stored at 1°C increased according to occurrence of chilling injury.

Major phospholipids in eggplant fruits were phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Contents of PE and PC in unripe eggplant fruits decreased apparantly, but overripe eggplant fruits showed slight decrease of PE and PC contents during storage at 1°C. Eggplant fruits at harvesting stage showed decrease of PE and PC contents during storage at 1°C and 20°C.

The rate of K ion leakage of eggplant fruit sections was increased by the addition of p-coumaric acid and chlorogenic acid. Pattern of K ion leakage induced by chlorogenic acid was similar to that induced by 2,4-dinitrophenol. Addition of ascorbic acid depressed the increase of K ion leakage induced by chlorogenic acid at 1°C.

Contents of PE and PC in eggplant fruit sections were decreased by the addition of chlorogenic acid at 1°C.

Ultrastructural changes of eggplant fruit sections, for example swelling of mitochondria and partial disappearance of tonoplast, were accelerated by the addition of chlorogenic acid at 1°C.

7. Studies on Nitrate and Nitrite in Horticultural Products Part VII. Nitrate and Nitrite Contents in Japanese Radish and the Changes during Growing, Storage, and Pickling Process. Akemi HATA (Faculty of Living Science, Kyoto Prefectural University) and Kuniyasu OGATA (Lab. Processing and Physiology of Horticultural Products) J. Food Sci. and Technol. (Japan). **25**, 280–286 (1978) (*in Japanese*)

Nitrate and nitrite contents and the activity of nitrate reductase of Japanese radish were analyzed during growing, storage and pickling with salt or salt-bran.

1. The varieties of radish used in this experiments were Minowase-spring type (sowing, April; harvesting, end of June), Minowase No. 2–summer type (sowing, August; harvesting, end of October), and Miyasige (sowing, September; harvesting, end of November).

2. Nitrate concentration in a root was higher toward the root tip and there were no significant difference in nitrate contents among cortex, vascular bundle and parenchyma at the middle part of the root.

3. Nitrate concentrations of the petioles, roots, and leaf blade were 1400–1600ppm, 700ppm, and 200–350ppm 40 days after sowing, respectively. During the growing stage from the above period to the harvesting date, nitrate contents of these parts decreased by about one-tenth of the initial ones. In Miyasige variety for which the times of fertilizing was increased, the decreasing rate for the period was about a half.

4. Nitrate reductase activity in Minowase-spring type was higher in leaf blade and lower

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according to the decreasing order of petioles and roots. During the growing stage, the activity increased in leaf blade, but were constant other two parts.

5. These three varieties of the radish roots were stored at 1 and 20°C. Changes of nitrate contents during the storage were not noted in both Minowase varieties, but in Miyasige, nitrate contents decreased slightly. Nitrate reductase activity were maintained at the low level during storage.

6. Changes of the amount of nitrate were observed through the process of pickling with salt-bran. After 26 days, the amounts became to be the equal level of nitrate in the roots and salt-bran. Maximum content of nitrite was only 0.4ppm in the process.

7. In the process of pickling with salt only, the changes of nitrate contents in the roots and brine both at 6 and 20°C were similar to those of pickling with salt-bran. Nitrite contents had maximum, 3 days after the start of pickling at 20°C (30ppm in the root and 230ppm in the brine), but the formation of nitrite at 6°C was suppressed markedly.

8. Studies on the Mechanism of Ethylene Action for Fruit Ripening Part IV. The Role of Glycolysis in Respiration of Banana Fruits Stimulated by Ethylene. Hirofumi TERAI and Kuniyasu OGATA. (Lab. processing and Physiology of Horticultural Products) J. Japan. Soc. Hort. Sci. **46**, 361–368 (1977) (*in Japanese*)

This study examines the role of glycolysis in respiration of banana fruits stimulated by ethylene.

Monoiodoacetate inhibited the respitation of banana peel sections in the absence of ethylene, and the inhibitory effect was strengthened in the presence of the gas (100μl/l). The experiment of incorporation of glucose-1-^{14}C, and glucose-6-^{14}C to carbon dioxide showed a conversion of the pathway from HMP to EMP with the advance of age in peel sections. But the period of alternation of the pathway did not coincide with that of the respiratory increase. However the incorporation rate of each penetrated glucose-^{14}C to carbon dioxide changed with the increase of the respiration rate of peel sections.

While the content of reducing suger increased continuously in pulp and peel, the levels of glycolytic intermediates such as G–1–P, G–6–P, F–6–P, F–1, 6–diP, dihydroxyacetone P and pyruvate corresponded to respiratory changes of climacteric in pulp tissue. Especially, the change of F–1, 6–diP content was remarkable among the intermediates. Although the respiration of peel sections decreased after removal of ethylene, the levels of G–6–P and F–6–P were higher in this tissue than in the tissue in which the respiration was continuously enhanced with ethylene. On the contrary, this tendency was reversed for F–1, 6–diP, dihydroxyacetone P and Pyruvate.

These result suggest that the respiration climacteric with ethylene may be related to the enhancement of glycolysis and the step between the F–6–P and F–1, 6–diP may be concerned with a regulation of this pathway in pulp and peel of banana fruits.

An alternation of temperature caused a change of respiration in green banana fruits. While the respiration was stimulated at 30°C, the levels of G–6–P and F–6–P decreased and other intermediates (F–1, 6–diP, dihydroxyacetone P, phosphoenolpyruvate, and pyruvate) had no change. This data indicates a difference of metabolism of glycolysis between the respiration climacteric and activated respiration by temperature alternation.

9. Studies on Biosynthesis of Esters in Fruit Volatiles Part IV. Oxidation of Added Alcohol to Acid in Banana Pulp, and Specificity of Acid in its Esterification.. Yoshinori UEDA and Kuniyasu OGATA (Lab. processing and Physiology of Horticultural Products) J. Food Sci. and Technol. (Japan). **25**, 440–455 (1978) (*in Japanese*)

When disks of banana pulp were incubated with vapor of alcohol (propyl–, iso-butyl–, butyl–, iso-amyl–, and amyl alcohol), they produced not only acetate ester of the alcohol, but also other ester with acids which were converted from added alcohols (propyl propionate, iso-butyl iso-butyrate, butyl butyrate, iso-amyl iso-valerate, and amyl valerate). These indirect esterification of added alcohols occurred strongly with straight-chain alcohols and weakly with branched alcohols. When straight-chain acids were added to the disks of banana pulp, conversion rate to esters was also higher than the rate of branched acids. Since both butyryl CoA and iso-butyryl CoA showed similar extent of esterification, the difficulty of conversion of branched chain acids to ester may be the step of acyl CoA formation. Above these results show one of the good explanation that banana volatile ester consist mainly of acetate and butyrate for acid moiety, iso-butyl alcohol and iso-amyl alcohol for alcohol moiety.

10. Studies on Biosynthesis of Esters in Fruit Volatiles Part III. Coenzyme A-dependent

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Esterification of Alcohols and Acids in separated Cells of Banana Pulp and its Homogenate. Yoshinori UEDA and Kuniyasu OGATA (Lab. processing and Physiology of Horticultural Products) J. Food Sci. and Technol. (Japan). 24, 624–630 (1977) (*in Japanese*)

This study was conducted to ascertain whether the formation of volatile esters in banana fruit would be a reverse reaction of esterase or a coenzyme dependent reaction. Esterase activity during ripening of banana fruit became higher with the increase of estrification of added alcohol and acid to banana slices. Esterase activity was, however, observed in green banana pulp, and vascular tissues from yellow banana pulp had fairly high capacity of esterification of added alcohol and acid, whereas esterase activity in the tissue was very low. Furthermore p-nitrophenyl phenylphosphonothionate (EPN), inhibitor of esterase, almost complately inhibited esterase activity from vascular tissues of banana pulp with low concentration, but the estrification in the tissue was not inhibited in the same concentrations.

Acetyl CoA and butyl alcohol changed to butyl acetate with very high extent in homogenate of pulp at over ripe stage. Acetyl CoA also made acetate ester in homogenate of pulp cells and in that of vascular tissues more distinctly. Homogenates and pulp cells could only produce acetate esters in the presence of acetyl CoA. Experiment with inhibitors also emphasized that acyl CoA is the real intermediate of esters of banana aroma.

11. Studies on Freezing Storage of Lateral Sprouts of Broccoli (Brassica oleracea var. Italica) Hiroyuki YAMANAKA and Kuniyasu OGATA Journal of Institute of Cold Chain 3, 107 (1977) (*in Japanese*)

This experiment was undertaken to determine the activity of peroxidase and the enzyme regeneration of broccoli in relation to ascorbic acid retention and quality of frozen broccoli.

1. The pH optima for peroxidase from flower buds and stems of raw broccoli were different, 4.6 and 4.1, respectively. The methods of blanching used were boiling water, steam and microwave. Microwave blanch was more effective on the inactivation of peroxidase than other methods. The residual peroxidase activity in stems were higher than in flower buds.

2. In raw or unblanched broccoli, peroxidase activity for flower buds declined after 5 months storage but the enzyme activity for stems remained higher. The regeneration of peroxidase in blanched broccoli was not observed during frozen storage at −20°C. The activity of inactivated peroxidase in broccoli tissue and crude enzyme solution were regenerated by holding at 20–35°C after blanching or after thawing of frozen materials, although no change in the activity of crude enzyme was found immediately after thawing.

3. Loss of ascorbic acid in broccoli was greater in water-blanched samples than steam– and microwave-blanched ones, but with water-blanched broccoli, the loss was less in stems than in flower buds and leaves. When the broccoli was held at room temperature after blanching, ascorbic acid of the sample blanched for 15 sec. was decreased but for 60 sec. was almost no change.

12. Physiological and Chemical Studies on Ascorbic Acid of Fruits and Vegetables. III. The Characteristics of Oxidized Ascorbate Reductase and Changes of the Enzyme Activity during Storage of Fruits and Vegetables. Naoki YAMAUCHI and Kuniyasu OGATA (Lab. Processing and Physiology of Horticultural Products) J. Jap. Hort. Sci., 47: 121–127 (1978) (*in Japanese*)

The changes of L-ascorbic acid (ASA) content during storage in fruits and vegetables depends on interaction between ascorbate oxidase (E.C.1.10.3.3) and peroxidase (E.C.1.6.5.4) which oxidize ASA and oxidized ascorbate reductase (E.C.1.6.5.4) which reduces oxidized ascorbate. The object of this experiment is to determine characteristics of oxidized ascorbate reductase and differences of this enzyme activities in several fruits and vegetables. We also investigated the changes of the activities during development of chilling injury (browning) and yellowing that a decrease of ASA content was remarkable.

The results obtained were as follows:

1. This enzyme catalyzed the reduction of L-monodehydroascorbic acid (MDHA) by means of NAD(P)H as a coenzyme and showed high activity in tris-HCl buffer.

2. The optimum pH and temperature of this enzyme were about 7.5–8.0 and 35°C, respectively.

3. This enzyme was extremely sensitive to SH reagents such as p-hydroxymercuribenzoate (PHMB), 5,5'-dithio-bis– (2–nitrobenzoic acid) (DTNB), and mono-iodoacetic acid and inhibited by chlorogenic acid. It was also inhibited by $AgNO_3$ (1×10^{-3} M, 1×10^{-4} M) and $FeCl_3$ (1×10^{-3} M), but other metal ions didn't actually exert on its activity.

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4. The activities of this enzyme were high in pumpkins, cucumbers, and carrots in which ascorbate oxidase activities were high, while in leaf vegetables, potatoes, and sweet potatoes its activities were low.

5. This enzyme activities during development of chilling injury in sweet pepper seeds and sweet potatoes in which ASA content decreased remarkably increased temporarily before occurrence of chilling injury and then declined with the advance of chilling injury.

6. The activity of this enzyme in spinach leaves lowered with the decrease of ASA content with yellowing.

13. **Some Physical and Chemical Properties of Pea Stem Necrosis Virus.** Tadao INOUYE, Takeshi OSAKI, Wataru NAKASONE and Kenichiro SHOHARA (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 43: 371 (1977) (*Abstract*).

14. **Serological Properties and Intracelluler Appearence of Pea Stem Necrosis Virus.** Tadao INOUYE, Takeshi OSAKI, Kenichiro SHOHARA, Wataru NAKASONE and Michiharu NAKANO (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 43: 372 (1977) (*Abstract*).

15. **Natural Hosts and a New Vector, *Bemisia lonicerae* , for the Virus Causing Yellow Dwarf Disease of Tomato.** Takeshi OSAKI*, Hirofumi KOBATAKE** and Tadao INOUYE* (*Lab. Plant Pathology, **Nara Agricultural Experimental Station) Ann. Phytopath. Soc. Japan 43:102 (1977) (*Abstract*).

16. **Seasonal Popuration Density of *Bemisia tabaci* and Transmission of Tobacco Leaf Curl Virus by Whiteflies.** Takeshi OSAKI*, Hirofumi KOBATAKE** and Tadao INOUYE* (*Lab. Plant Pathology, **Nara Agricultural Experimental Station) Ann. Phytopath. Soc. Japan 43: 102 (1977) (*Abstract*).

17. **Application of Simplified Double Diffusion Techniques to Detection of Viruses Infecting Pepper Plants.** Takeshi OSAKI, Kenichiro SHOHARA, Fumihiro TERAMI and Tadao INOUYE (Lab. Plant Pathology) Proc. Kansai Plant Protection Soc. 20: 97–98 (1978).

18. **Dimer Type Particles Associated with Yellow Dwarf Disease of Tomato and Leaf Curl Disease of Tobacco.** Takeshi OSAKI and Tadao INOUYE (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 44: 96 (1978) (*Abstract*).

19. **Resemblance in Morphology and Intranuclear Appearence of the Viruses Isolated from Yellow Dwarf Diseased Tomato and Leaf Curl Diseased Tobacco.** (Lab. Plant Pathology) Takeshi OSAKI and Tadao INOUYE Ann. Phytopath. Soc. Japan 44: 167–178 (1978) (*in Japanese*).

20. **Serological Properties of Tobacco Leaf Curl Virus. I.** Takeshi OSAKI and Tadao INOUYE (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 44: 385 (1978) (*Abstract*).

21. **Two Plant Species as Possible Carriers of Spotted Wilt Disease of Tomato Occurring in Japan.** Hirofumi KOBATAKE*, Takeshi OSAKI** and Tadao INOUYE** (*Nara Agricultural Experimental Station, **Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 44: 386 (1978) (*Abstract*).

22. **Two Flexuous-rod Viruses Isolated from Garlic.** Young LEE, Shozo YAMAZAKI, Takeshi OSAKI and Tadao INOUYE (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 44: 384 (1978) (*Abstract*).

23. **Host Reactions of Mechanically Transmissible Legume Viruses of the Northern Temperate Zone.** R. HAMPTON, L. BECZNER, D. HAGEDORN, L. BOS, T. INOUYE, O. BARNETT, M. MUSIL and J. MEINERS Phytopathology 68: 989–997 (1978).

24. **Simplified Techiques for Serodiagnosis of Plant Viruses. I. Simplified Radial Diffusion and Double Diffusion Techniques for Detection of CMV.** Kenichiro SHOHARA and Tadao INOUYE (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan. 44: 97 (1978) (*Abstract*).

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25. Simplified Techniques for Serodiagnosis of Plant Virusess. II. Detection of CMV from Various Plants Infected with CMV. Tadao INOUYE and Kenichiro SHOHARA (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 44: 97 (1978) (*Abstract*)

26. Host Range and Intracelluler Appearence of Thread-like Particles (Bd-F) Isolated from Burdock. Michiharu NAKANO and Tadao INOUYE (Lab. Plant Pathology) Ann Phytopath. Soc. Japan 44: 96 (1978) (*Abstract*).

27. Serological Relationships Between Broad Bean Necrosis Virus and the Viruses of Tobamovirus Group. Wataru NAKASONE and Tadao INOUYE (Lab. Plant Pathology) Ann. Phytopath. Soc. Japan 44: 97 (1978) (*Abstract*).

28. *Pythium* spp. Causing Damping-off and Rhizome Rot of Zinger. Takio ICHITANI and Toshinori SHINSHU (Lab. Plant Pathology & Nagasaki Agr. Exp. Sta.). Ann. Phytopath. Soc. Japan 43: 337–338 (1977) (*Japanese abstr.*).

Pythium ultimum other than *Pythium zingiberum* was only isolated from Azuma and Moriyama areas in Nagasaki prefecture, Japan in 1975. *P. ultimum* was however, doubtfully pathogenic, whereas *P. zingiberum* was strongly pathogenic.

29. *Pythium Porphyrae* Takahashi et Sasaki, sp. nov. Causing Red Rot of Marine Red Algae *Porphyra* spp. Minoru TAKAHASHI, Takio ICHITANI and Minoru SASAKI (Fukuda, Sakai, Lab. Plant Pathology & Tohoku Regional Fisheries Res. Lab.). Trans. mycol. Soc. Japan 18: 279–285 (1977).

The fungus causing red rot of *Porphyra yezoensis* Ueda is isolated from the diseased thalli in the coastal waters of northeastern Japan and highly pathogenic. To this fungus, a new name *Pythium porphyrae* Takahashi et Sasaki, sp. nov. was given.

30. Development of Rhizome Rot of Zinger in Vinyl House. Takio ICHITANI & Tadamitsu BANDO (Lab. Plant Pathology & Wakayama Agr. Ext. Sta.). Ann. Phytopath. Soc. Japan 44: 86 (1978) (*Japanese abstr.*).

Development of rhizome rot of zinger was followed under vinyl house conditions and high probability to seed transmission of the causal fungus was discussed.

31. Fine Structure of *Pythium aphanidermatum* Oospores. Yoshiaki CHIKUO, Takio ICHITANI and Yoshiya TAGAMI (Lab. Plant Pathology). Ann. Phytopath. Soc. Japan 44: 89 (1978) (*Japanese abstr.*)

Both young oogonium and oospores with different stages were observed by electron microscope, and the relationships between the stages of oospores and the thickness of oospore walls were discussed.

32. Isolation of *Pythium zingiberum* from Various Parts of Rotted Zinger Rhizome and Its Cultivated Land at Different Districts. Takio ICHITANI & Toshinori SHINSHU (Lab. Plant Pathology & Nagasaki Agr. Exp. Sta.). Ann. Phytopath. Soc. Japan 44: 369–370. (1978) *Japanese abstr.*).

Pythium zingiberum was considered to be the main causal fungus to rhizome rot of zinger in Japan.

33. Stimulation of the Growth of Excised Cultured Roots of Soya Bean by Abscisic Acid. Toshihiko YAMAGUCHI and H.E. STREET (Lab. Crop. Sci. and the Late Prof. of Bot. Labs., Leicester Univ., U.K.) Ann. Bot. 41, 1129–1133 (1977).

The percentage germination of soybean seed declines quickly and there is an increase in the incidence of abnormal germination during the storage. On the hypothesis that loss of capacity for normal germination might be related to changes in plant growth regulator balance within the seed, it was decided to study the growth in culture of excised root tips from new and stored seed and their responses to externally applied growth regulators.

During the course of this work a stimulation of excised root growth by abscisic acid (ABA) was discovered. Applications of ABA (0.01–0.001 mg/l) during the first two culture passages enhanced the linear growth of the main axis, the number of emergent laterals and the total length of laterals.

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This effect has been shown with roots derived from seed in different period of storage.

It is clear that ABA can act as a growth promoter and that its action probably involves its interaction with other endogenous growth regulators. The persistent and highly significant enhancement of the growth of cultured soybean roots by ABA, an effect which involves both cell division and cell extension, appears to be an excellent system for further study of its mechanism of action as a growth promoter and particularly for study of its interaction with other natural growth regulators. Such studies could also provide valuable information regarding the changes in the growth regulator levels which proceed during the storage of soybean seeds.

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Section II

1. On the Soil Borne Desease and the Avoiding Methods – Studies on the Engineering Problems of Soil Sickness in Greenhouse Production (3) – Yoshihiko OGINO (Lab. Utilization of Water in Agr.) Transaction of The Jap. Soc. of Irrigation, Drainage and Reclamation Eng. No. 73 (1977) (*in Japanese*)

1) It is found that one of the causes of the soil sickness unknown until now is soil borne diseases.

2) The ways of avoiding these diseases consists in flooding treatment, chemical sterilization, and steam sterilization.

3) As a result of technical analysis, the following conditions must be considered: ① efficiency of the treatment, ② term of the trearment, ③ costs of the treatment.

4) On the other hand, the commercial conditions are analyzed, then it is found that the cropping system and the shipping system must be taken into consideration in choosing these ways.

2. On the Utilization of Water in the Case of Different Depth of Soil Layer at the Irrigation Start and the Irrigation - Studies on the Available Utilization of Rainfall in the Field Irrigatioon (III) - Katsuhiko YABE (Lab. Utilization of Water in Agr.) Transactions of The Jap. Soc. of Irrigation, Drainage and Reclamation Eng. No. 75 (1978) (*in Japanese*)

For the purpose to study the utilization of water, experiments were executed by setting the various water depth at irrigation and irrigation start, namely 10 cm–10 cm, 15 cm–20 cm, 15 cm–30 cm and 20 cm–30 cm plots using Field Soil of University of Osaka Prefecture.

The results obtained were as follow;

First, the significant difference was recognized between 20 cm–30 cm and 15 cm–30 cm plots and between 10 cm–10 cm and 15 cm–20 cm in respect of total water quantity. And also the significant difference was recognized between 25 cm–20 cm plot and other three plots with respect to available rainfall.

Secondly, the significant difference was not recognized with respect to harvest. But the difference was recognizd aboud 10% between 10 cm–10 cm and 20 cm–30 cm plots and between 15 cm–20 cm and 15 cm–30 cm.

Thirdly, the mean evapotranspiration on water balance was about 8.0 mm/day in 10 cm–10 cm, about 8.3 in 15 cm–20 cm, about 7.0 in 15 cm–30 cm and about 6.4 in 20 cm–30 cm. And as water quantity increased, more amount of soil moisture was consumed without connection to harvest of these plots.

Last, judging from these experimental results, it was clarified that 20 cm–30 cm plot can save much water quantity, store and utilize much rainfall, and also introduces much harvest and smaller evapotranspiration.

3. About the Utilization of Water on the Various Application Method of Irrigation Water under the Same Climate Condition - Studies on the Utilization of Available Rainfall in the Field Irrigation (IV) - Katsuhiko YABE (Lab. Utilization of Water in Agr.) Transactions of the Jap. Soc. of Irrigation, Drainage and Reclamation Eng. No. 76 (1978) (*in Japanese*)

For the purpose of studying the utilization of available rainfall and the effect utilization of water quantity, experiments were practised with setting the various water depth per irrigation and the various depth of irrigation start, namely 10 cm–10 cm, 10 cm–20 cm 10 cm–30 cm 20 cm–20 cm, 20 cm–30 cm, 30 cm–30 cm and non-irrigation section, by using Osakafudai Field Soil.

The results obtained were as follows;

Firstly, the purposive difference was recognized with regard to water quantity, available rainfall, and harvest.

Secondly, the more available rainfall increased, the more water quantity decreased. And the single-valued relation existed in between available rainfall and water quantity, and its correlation was cleared to be very high.

Thirdly, in case of the shallower depth of irrigation start such as 10 cm–10 cm or 10 cm–20 cm section that the period is short from irrigation to next and the deeper water depth per irrigation such as 20 cm–20 cm or 30 cm–30 cm section that the period is long, the utilization rate of available rainfall or the effective utilization rate of water is apt to be depended on rainfall pattern or timing. Because the former is much numbers of irrigation and apt to dry right away at the shallower soil layer, the latter is much water quantity per irrigation and high moisture content at the deeper soil layer.

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Fourthly, 20 cm–30 cm section was judged as the best irrigation method of all thes experimental results from utilizing rainfall availably, having the effect of saving irrigation water, being expected to utilize effectively soil moisture at the deeper soil layer and not being depended on the climate conditions so much.

4. Investigation on Soil Hydrology in Reclaimed Land Used as Open Space. Sanji TEJIMA and Kazuo MURASHIMA (Lab. Utilization of Water in Agr.) Tech. Rep., Nambu Park Office of Osaka Pref. (1978)

5. On the Characteristics of Field Irrigation by Using Porous Ceramic Tubes. Zenji KATO, Tsutomu NISHIIDE (Faculty of Agr., Gifu Univ.), Kazuo MURASHIMA and Sanji TEJIMA (Lab. Utilization of Water in Agr.) Reported at Annual Meeting of The Kyoto Branch of The Jap. Soc. of Irrigation, Drainage and Reclamation Eng. (1977)

6. An Investigation of the Pressure Membrane Method for Measuring pF-Moisture Properties of Soil. Akira TAKAYANAGI, katsuhiko YABE and Sanji TEJIMA (Lab. Utilization of Water in Agr.) Reported at Annual Meeting of The Kyoto Branch of The Jap. Soc. Irrigation, Drainage and Reclamation Eng. (1977)

7. On Cultivation Experiments by Using Porous Ceramic Tubes-Experimental Studies on Sub-Irrigation II – Zenji KATO, Tsutomu NISHIIDE (Faculty of Agr., Gifu Univ.), Kazuo MURASHIMA and Sanji TEJIMA (Lab. Utilization of Water in Agr.) Reported at Annual Meeting of The Jap. Soc. of Irrigation, Drainage and Reclamation Eng. (1978)

8. On the Characteristics of Infiltration and Runoff Recession in a Small Basin. Kazuo MURASHIMA, Toshiyuki TSUJI, Yoshinobu MATSUSHIMA and Sanji TEJIMA (Lab. Utilization of Water in Agr.) Reported at Annual Meeting of The Jap. Soc. of Irrigation, Drainage and Reclamation Eng. (1978)

9. Investigation on Change of Total Discharge of Pumping Drainage under the Impact of the Urbanization. Kiyoshi SAKAI (Faculty of Agr., Gifu University) and Yoshihiko OGINO (Lab. Utilization of Water in Agr.) Reported at Annual Meeting of The Jap. Soc. of Irrigation, Drainage and Reclamation Eng. (1978)

10. Case Studies of the Management of Agricultural Water Use under the Impact of the Urbanization. Yoshihiko OGINO (Lab. Utilization of Water in Agr.) and Masami OKAMOTO (Faculty of Agr. University of Tokyo) Reported at Annual Meeting of The Jap. Soc. of Irrigation, Drainage and Reclamation Eng. (1978)

11. About the Available Rainfall and Water Consumption on the Various Application Method of Irrigation Water. Katsuhiko YABE (Lab. Utilization in Agr.) Reported at Annual Meeting of The Jap. Soc. of irrigation, Draingae and Reclamation Eng. (1978)

12. Studies of the Engineering Problems of Soil Sickness in the Greenhouse Production. Yoshihiko OGINO (Lab. Utilization of Water in Agr.) Doctor Thesie (1977)

13. Studies on the Heater Noise of Vinyl Greenhouse Part I. Noise Characteristics. Shigeo UMEDA, Kentaro MOHRI (Lab. Agricultural Machinery) and Toshio MURATA, Shinroku MORITA (Osaka Agricultural Research Center) J. Agr. Mach. (Japan) 39-4 497-503 (1978) (*in Japanese*)

This paper describes the noise distribution and its characteristics of the hot air heater used in vinyl greenhouse.

It is a greenhouse having an overall length of 40.5 m, a width of 10 m, and a height of 4.2 m. The heater is a hot air type with axial flow fan having a flow rate of 60 m^3/min, and gun type burner having a quantity of heat of 52,000 Kcal/hr. The noise sourse is an axial flow fan attached to the heater.

The noise level in greenhouse is 98–67 dB (A) within about 10 m from the heater, but less than 55 dB (A) at the opposite end of greenhouse. The noise level at the outside of the greenhouse is over 50 dB (A) within 20 m from the greenhouse.

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The high frequency noise descreased with the distance from greenhouse, but the noise of 200–300 Hz caused by axial flow fan remains high. The effect of noise reduction by vinyl film is very little.

14. Studies on the Heater Noise of Vinyl Greenhouse Part II. Reduction of Noise. Shigeo UMEDA, Kentaro MOHRI (Lab. Agricultural Machinery) and Toshio MURATA, Shiroku MORITA (Osaka Agricultural Research Center) J. Agr. Mach. (Japan) **40**–1 99–104 (1978) (*in Japanese*)

The noise caused by hot air heater in vinyl greenhouse is a matter of increasing concern in previous report. To reduce the noise from the heater, a sound proof wall was designed. It consisted of the perforated wooden plate, air layer and sound absorption materials (urethane foam and chaffs).

As a hot air heater circumscribed by the sound proof walls, its effect of absorption was measured in same condition of previous experiment.

As a result, the noise could be reduced about 7–9 dB (A) in greenhouse, and the noise around the greenhouse became under 50 dB (A) at the distance 15–20 m from the greenhouse.

It also became clear that the chaffs could be use as effective sound absorption materials.

15. Hail Observation in Gunma Prefecture, 1971–1975. Yukio OMOTO (Lab. Agrometeorological Environment) and Turuhei YAGI, Hiroshi SEINO, Tsuneharu YONETANI (National Research Center for Disaster Prevention) J. Agr. Meteorology (Tokyo). **34**, 17–21, (1978) (*in Japanese*)

16. On Relationships between Hailfall Characteristics and Crop Damage. Yukio OMOTO (Lab. Agrometeorological Environment) and Hiroshi SEINO (National Research Center for Disaster Prevention) J. Agr. Meteorology (Tokyo). **34**, 65–76, (1978) (*in Japanese with English summary*)

17. On the Thermal Diffusivity of Soil. Shiro KANESEKI (Lab. Agrometeorological Environment) J. Agr. Meteorology (Tokyo). **34**, 13–16, (1978) (*in Japanese*)

18. Some Agro-Climatological Consideration on the Distribution of Food Grain Sorghum (*Sorghum bicolor* MOENCH) (1). Shirou HASEGAWA (Lab. Agrometeorological Environment) Jap. J. Trop. Agr., **21**, 134–137, (1978)

19. Agro-Climatological Studies on C_3-Plants and C_4-Plants. (3). Transpiration rates and Leaf Temperatures. Shirou HASEGAWA (Lab. Agrometeorological Environment) J. Agr. Meteorology (Tokyo), 33 129–136, (1977) (*in Japanese with English summary*)

20. Climate and Agriculture of Taiwan and South Korea. Shirou HASEGAWA (Lab. Agrometeorological Environment). J. Agr. Meteorology (Tokyo), **34**, 95–99, (1978) (*in Japanese*)

21. Remote Sensing of Plant Activity by Infrared Colour Photograph. Kazutoshi YABUKI and Masatoshi AOKI (Lab. Environmental Control in Biology) Presented at the Simposium of the Japanese Society of Environmental Control in Biology. (November, 1977, Tsukuba) (*in Japanese*)

22. Characteristics of the Forest Microclimate. Kazutoshi YABUKI, Masatoshi AOKI (Lab. Environmental Control in Biology) and Ken HAMOTANI (Lab. Agrometeorological Environment) In "JIBP Synthesie Volume 18, Biological Production in a Warm-temperate Evergreen Oak Forest of Japan" (Ed. by T. KIRA, Y. ONO and T. HOSOKAWA). University of Tokyo Press, Tokyo, 55–64 (1978)

23. The Effect of Wind Speed on the Photosynthesis of Rice Field. Kazutoshi YABUKI and Masatoshi AOKI (Lab. Environmental Control in Biology) In "JIBP Synthesis Volume 19, Ecophsiology of Photosynthetic Productivity" (Ed. by M. MONSI and T. SAEKI). University of Tokyo Press, Tokyo, 152–159 (1978)

24. Studies on the Effects of Wind Speed on Photosynthesis. (6) The Relation between Wind Speed and Diffusive Resistance of Cucumber Leaves. Kazutoshi YABUKI and Makoto KIYOTA (Lab. Environmental Control in Biology) J. Agr. Met. (Japan). **34**, 59–63 (1978) (*in Japanese with English summary*)

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25. Studies on the Effects of Wind Speed on Photosynthesis. (7) The Structure of Boundary Layer near Leaf Surface (II). Kazutoshi YABUKI and Yoshinobu HARAZONO (Lab. Environmental Control in Biology) J. Agr. Met. (Japan). **34**, 87–94(1978) (*in Japanese with English summary*)

26. High Efficiency Production of Starch with Single Leaf and Node Cuttings Culture of Sweet Potatoes. Kazutoshi YABUKI (Lab. Environmental Control in Biology) and Tsutomu UEWADA (Lab. Agrometeorological Environment) Presented at the International Symposium on Potential Productivity in Protected-Cultivation. (August 29 and 30, 1978, Kyoto)

27. Studies on the Carbon Dioxide Enrichment for Plant Growth, VII. Changes in Dry Matter Production and Photosynthetic Rate of Cucumber during Carbon Dioxide Enrichment. Masatoshi AOKI and Kazutoshi YABUKI (Lab. Environmental Control in Biology) Agr. Met., **18**, 475–485 (1978)

28. A Method to Measure the Dynamic Response of Net Photosynthetic Rate in the Controlled- Environment Chamber. Masatoshi AOKI (Lab. Environmental Control in Biology) J. Agr. Met. (Japan). **34**, 1–6, (1978) (*in Japanese with English summary*)

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Section III

1. A Possible Role of Allantoin and the Influence of Nodulation on its Production in Soybean Plants. Shinsuke FUJIHARA, Katsumi YAMAMOTO and Masuro YAMAGUCHI (Lab. Soil Sci. and Plant Nutri.) Plant and Soil, 48, 233–242 (1977)

To examine the influence of nodulation on the production of nitrogenous compounds, soybean plants (*Glycine max* var. Tamanishiki) were grown with or without N-fertilizer in the field, and the changes in amino-N and allantoin-N content in the different organs were determined throughout the growth period. In the stem allantion-N markedly increased after the flowering period and then decreased during seed formation. Allantoin accumulated in the pod (up to 70 per cent of total alcohol soluble-N) during pod formation, while in the seed the main N-compounds were amino acids, the allantoin concentration being very low. In well-nodulated soybeans grown without N-fertilizer, allantoin content in every plant organ was always high compared to beans grown with N-fertilizer, but amino-N content was comparatively low.

Another experiment, in which soybean plants were allowed to form nodules by growing on a N-free medium, and in which a fixed-N supply was then controlled by the addition of various levels of ammonium, was made in a greenhouse. When nodules were formed, the subsequent addition of high concentrations of ammonium caused the accumulation of allantoin as well as the accumulation of amides and arginine. A possible role of allantoin and some aspects of its production in soybeans are discussed.

2. Participation of Iron in Denitrification in Waterlogged Soil Yasuhiko KOMATSU, Masanosuke TAKAGI* and Masuro YAMAGUCHI (Lab. Soil Sci. and Plant Nutri, *Lab. Biophysical Chem.) Soil Biol. Biochem, 10, 21–26 (1978)

A study was made of the formation of anaerobiosis in a waterlogged soil. A dilute soil suspension containing NO_3^-, Fe^{3+}, sodium citrate, a limited amount of 0_2, and trace elements was used as a model of waterlogged soil. Polarography was used to detect dissolved 0_2, Fe^{3+} and Fe^{2+}. The fates of the NO_3^- and Fe^{3+} during and after 0_2 consumption by the microorganisms were studied in a specially designed vessel. A close correspondence was obtained between the reduction of NO_3^-, NO_2^-, and Fe^{3+} and the growth of denitrifying bacteria in the close system employed. From the experimental results we presume that microorganisms which respire NO_3^- are also capable of utilizing Fe^{3+} in their respiration. The mechanisms of the reduction of these chemical species by the microorganisms are also discussed, emphasising the possibility of the participation of chemical reduction of NO_2^- by Fe^{2+} in the overall reduction process.

3. Probable Site of Allantoin Formation in Nodulating Soybean Plants. Shinsuke FUJIHARA and Masuro YAMAGUCHI (Lab. Soil Sci. and Plant Nutri.) Phytochemistry, 17, 1239–1243 (1978)

Nodulated soybean plants contain high concentration of allantoin in all parts. Excision of nodules from the roots brought about a marked decrease in allantoin. To examine the function of nodules in allantoin production, nodulated and nodule-detached soybeans were fed with $^{15}NH_3$ for 1 week. High abundance of ^{15}N was found in the amino acid-N fraction of both plants. In the root and stem of the nodulated plants, ca 80% of the nitrogen in this fraction was derived from the NH_3 added in the medium. Excess ^{15}N was detected also in allantoin-N fraction, but the ^{15}N content was very low in contrast to that in amino acid-N fraction. The site involved in the allantoin formation and the possible significance of its synthesis are discussed in relation to symbiotic nitrogen fixation.

4. Effects of Allopurinol (4-Hydroxypyrazolo (3,4-d) Pyrimidine) on the Metabolism of Allantoin in Soybean Plants. Shinsuke FUJIHARA and Masuro YAMAGUCHI (Lab. Soil Sci. and Plant Nutri.) Plant Physiology, 62, 134–138 (1978)

Some studies on the effects of xanthine oxidase inhibitor allopurinol (4-hydroxypyrazolo (3,4-d) Pyrimidine) on allantoin metabolism of soybean plants (*Glycine max* cv. Tamanishiki) are reported. Soybean seedlings, aseptically germinated for 96 hours on agar containing 1 millimolar allopurinol, contained only slight amounts of allantoin, allantoic acid, and urea as compared with controls. Analysis of purine and pyrimidine of the allopurinol-treated seedlings showed marked accumulation of xanthine both in the cotyledons and seedling axes. No hypoxanthine accumulation was found. Xanthine accumulation due to allopurinol treatment was relatively low after the cotyledons had fallen. For nodulated plants allopurinol caused a significant drop in allantoin (+ allantoic acid) in the stems and nodules accompanied by a striking accumulation of xanthine in the

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nodules. The xanthine concentration in the nodules far exceeded that in the germinated seedlings. Allopurinol at a concentration of 50 micromolar strongly inhibited xanthine oxidase prepared from soybean nodules.

The results suggested that the main pathway of allantoin formation in soybean plants was through purine decomposition, via xanthine-uric acid. It was specially noted that a very active purine-decomposing system existed in soybean nodules.

5. Fate of Deuterium Labelled Amino Acid in the Scutellum of Corn Seeds during Germination. Kisaburo SAMUKAWA and Norimi AKIMORI* (Lab. Soil Sci. and Plant Nutri., * Application Laboratory, Naka Works, Hitachi Ltd.,) Radioisotopes **26**, 891–894 (1977)

6. Measurement of Relative Isotopic Abundance of Nitrogen–15 in the Amino Acids by a GC–MS Technique. Kisaburo SAMUKAWA, Norimi AKIMORI* and Masuro YAMAGUCHI (Lab. Soil Sci. and Plant Nutri., * Application Laboratory, Naka Works, Hitachi Ltd.,) J. Sci. Soil Manure Japan **49**, 383–388 (1978)

A study of the gas chromatographic mass spectrometer assay (GC–MS) for the relative isotopic abundance of ^{15}N-enriched amino acids has been carried out. A Hitachi gas mass (RMU–6MG) was used to determine the relative isotopic abundance of ^{15}N in the mass fragments of amino acid peaks separated by GC.

1) GC–MS assay for the relative isotopic abundance of ^{15}N-enriched aspartic acid (50 atom % excess ^{15}N) was in good agreement with the guaranteed content of ^{15}N.

2) The relative isotopic abundances of some natural amino acids were measured with GC–MS. Their variation coefficents were in the range of about 1 to 2 and were reproducible.

3) The relative isotopic abundance of ^{15}N-enriched amino acids in the roots and shoots of corn seedlings germinated in a $K\,^{15}NO_3$ solution was measured, and no evidence for the incorporation of ^{15}N into amino acids was recognized. Therefore, it was comfirmed that the nitrogen in α-amino groups of free amino acids present in the roots and shoots within 72 hours after germination was not dependent on exogenous nitrogen source of nitrate nitrogen.

7. Land Classification Map, Osaka Prefecture (scale 1:100,000) – Soil Map, Land-use Capability Classification Map, Soil Productive Capability Classification Map and Related Expositions. Yutaka KOMAI, Ryuichiro DEURA (Lab. Soil Sci. and Plant Nutr.) Shuichi YOSHIMURA (Osaka Agricultural Research Center) and Hisao FURUKAWA (Faculty of Agri., Kyoto Univ.) Published by Land Bureau, National Land Agency (1976)

Soils over the entire prefectural area were classified into the soil groups currently recognized for general use and standardized by the National Land Agency. Studies were carried out under field survey, examination of aerial photographs, determination of soil distribution area and investigation on related data. Classification of soils and characterization of their distributions and productivities were described and illustrated with fifty tables and three maps.

8. Basic Investigations of Land Grouping in Senshu District – On the Classification, Distribution and Productive Capability of Soils. Yutaka KOMAI and Ryuichiro DEURA (Lab. Soil Sci. and Plant Nutr.) Published by the Planing Project Division of Osaka Pref. (1976) (*in Japanese*)

9. Investigation of the Natural Conditions on accout of Devising the Basic Design of Misaki Multiple Purpose Planning. Yutaka KOMAI and Ryuichiro DEURA (Lab. Soil Sci. and Plant Nutr.) Tech. Rep., Misaki Town Office of Osaka Pref. (1976) (*in Japanese*)

10. Basic Investigations of Land Grouping in Kita-Osaka District – On the Classification, Distribution and Productive Capability of Soils. Yutaka KOMAI and Ryuichiro DEURA (Lab. Soil Sci. and Plant Nutr.) Published by the Civil Engineering Division of Osaka Pref. (1977) (*in Japanese*)

11. Soil Groups and their Distribution in Osaka Prefecture. Yutaka KOMAI and Ryuichiro DEURA (Lab. Soil Sci. and Plant Nutr.) Bull. Univ. Osaka Pref. Ser. B, Vol. 33 (1978)

12. Hydrolysis of Phenyl α-Maltoside Catalyzed by Saccharifying α-Amylase from Bacillus subtilis. Kiyoshi ISHIKURA, Yasunori NITTA and Takehiko WATANABE (Lab. Biophys. Chem), J. Biochem., **81**, 1187–1192 (1977)

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13. Polarography of α-Keto Acids in Aqueous and Nonaqueous Solutions. Mitsuaki OHMORI and Masanosuke TAKAGI (Lab. Biophys. Chem.), Bull. Chem Soc. Japan, **50**, 773–778 (1977)

14. Investigations on Polarographic *o*-Phenylenediamine Method for Microdetermination of Vitamin C. Mitsuaki OHMORI, Hachiro AKEHASHI, Shigeru SHIGEOKA, Shozaburo KITAOKA and Masanosuke TAKAGI (Lab. Biophys. Chem.), Bunseki Kagaku, **26**, 56–60 (1977) (*in Japanese*)

15. Biochemical Calorimetry (I) – Calorimetry Related to Biochemical Functions (part 1). Harumi FUKADA and Katsutada TAKAHASHI (Lab. Biophys. Chem.), Calorimetry and Thermal Analysis, **4**, 67–75 (1977) (*in Japanese*)

16. Biochemical Calorimetry (I) – Calorimetry Related to Biochemical Functions (part 2). Harumi FUKADA and Katsutada TAKAHASHI (Lab. Biophys. Chem.), Calorimetry and Thermal Analysis, **4**, 117–125 (1977) (*in Japanese*)

17. Biochemical Calorimetry (II) – Thermodynamic Functions of Proteins. Katsutada TAKAHASHI and Harumi FUKADA (Lab. Biophys. Chem.), Calorimetry and Thermal Analysis, **4**, 165-171 (1977) (*in Japanese*)

18. A Facile Preparation of Dehydro-L-ascorbic Acid-Methanol Solution and Its Stability. Mitsuaki OHMORI and Masanosuke TAKAGI (Lab. Biophys. Chem), Agr. Biol. Chem., **42**, 173–174 (1978)

19. Difference Spectroscopic Study of the Interaction between Taka-amylase A and Substrates. Toshiko KUNIKATA, Yasunori NITTA and Takehiko WATANABE (Lab. Biophys. Chem.), J. Biochem., 83, 1435–1442 (1978)

20. Biochemical Calorimetry (III) – Measurement of Cellular Systems. Katsutada TAKAHASHI and Harumi FUKADA (Lab. Biophys. Chem.), Calorimetry and Thermal Analysis, **5**, 34–44 (1978) (*in Japanese*)

21. Biochemical Calorimetry (IV) – Calorimetry as an Analytical Tool. Katsutada TAKAHASHI (Lab. Biophys Chem.), Calorimetry and Thermal Analysis, **5**, 66–76 (1978) (*in Japanese*)

22. Asymmetric Synthesis of α-Methylornithine. Mitsunori KIRIHATA, Sei MIHARA, Itsuo ICHIMOTO and Hiroo UEDA (Lab. Pesticide Chem.) Agric. Biol. Chem. **42**, 185–186 (1978)

23. Asymmetric Synthesis of 4-Amino-3-hydroxy-6-methylheptanoic Acid (AHMHA). Mitsunori KIRIHATA, Hitoshi TOKUMORI, Itsuo ICHIMOTO and Hiroo UEDA (Lab. Pesticide Chem.) Nippon Nogeikagaku Kaishi, **52**, 135–141 (1978) (*in Japanese*)

24. Reduction of β-Ketoamino Acid. Mitsunori KIRIHATA, Hitoshi TOKUMORI, Itsuo ICHIMOTO and Hiroo UEDA (Lab. Pesticide Chem.) Nippon Nogeikagaku Kaishi, **52**, 271–276 (1978) (*in Japanese*)

25. Synthesis of New Pyrazine Compounds from Diaminomaleonitrile. Tadataka TSUDA and Hiroo UEDA (Lab. Pesticide Chem.) Nippon Nogeikagaku Kaishi, **52**, 213–217 (1978) (*in Japanese*)

26. Studies on the Structure of Mesityloxide Dimer. Osamu NAKANISHI, Itsuo ICHIMOTO and Hiroo UEDA (Lab. Pesticide Chem.) Nippon Nogeikagaku Kaishi, **52**, 373–378 (1978) (*in Japanese*)

27. Overcrowding Factors of Mosquito Larvae X. Structure-Bioactivity Relationship and Bacterial Activation of the Alkyl-branched Hydrocarbons. Itsuo ICHIMOTO, Toshikazu ONO, Yoshinobu NAOSHIMA, Hiroo UEDA and Toshiaki IKESHOJI (Lab. Pesticide Chem.) Appl. Ent. Zool., **12**, 265–273 (1977)

28. On the Antibacterial Substances in the Roots of *Solidago altissima L.* Hisao TSUJI, Yumi TANI and Hiroo UEDA (Lab. Pesticide Chem.) Nippon Nogeikagaku kaishi, **51**, 609–615

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(1977) (*in Japanese*)

29. Isolation of β-Amyrin, α-Spinasterol and α-Spinasteryl Glucoside from the Roots of *Solidago altissima L.* Hisao TSUJI, Yumi TANI and Hiroo UEDA (Lab. Pesticide Chem.) Nippon Nogeikagaku Kaishi, 52, 49–50 (1978) (*in Japanese*)

30. On the Antibacterial Properties of Kolavenic Acid isolated from the Roots of *Solidago altissima L.* Hisao TSUJI, Yumi TANI and Hiroo UEDA (Lab. Pesticide Chem.) Bōkin-Bōbai-shi, 6, 22–24 (1978) (*in Japanese*)

31. Isolation of Indolmycin and Its Derivative as Antagonists of L-Tryptophan. Akira HIROTA, Yuji HIGASHINAKA and Heiichi SAKAI (Lab. Natural Products Chemistry) Agric. Biol. Chem. 42, 147–151. (1978)

Antagonists of *L*-tryptophan, 927-B substance (I) and 927-A substance (II) were isolated from the culture filtrate of No. 927 strain, which was identified as *Streptomyces griseus*. I was identified as indolmycin [(−)-2-methylamino-5-α-(3'-indolyl)ethyl-2-oxazolin-4-one] and II was structurally determined to be N-demethyl-C-demethyl-indolmycin. II has hitherto been unknown. Addition of *L*-tryptophan to a minimal medium weakened the antibacterial activity of I., while that of *D*-tryptophan strengthened the activity.

32. Polylysine Produced by *Streptomyces*. Shoji SHIMA and Heiichi SAKAI (Lab. Natural Products Chemistry) Agric. Biol. Chem. 41, 1807–1809 (1977) (Short Communication)

33. Isolation and Structure Elucidation of I-851, an Antagonist of Chloramphenicol. Yoshitaka IMAGAWA, Shoji SHIMA, Akira HIROTA and Heiichi SAKAI (Lab. Natural Products Chemistry) Agric. Biol. Chem. 42, 681–682 (1978) (Short Communication)

34. Studies on FBP-ase of *Phodotorula glutinis*– Purification and Properties. Masahira NAKAGAWA, Yoshiharu NUNOUCHI and Heiichi SAKAI (Lab. Natural Products Chemistry) Presented at the Annual Meeting of Agricultural Chemical Society of Japan. (Apr. 2, 1978, Nagoya) Abst. p. 113

35. On a Substance isolated from *S. aureus* Culture added by 7-Iodo-Kojic Acid. Takashi KOTANI and Heiichi SAKAI (Lab. Natural Products Chemistry) Presented at the Annual Meeting of Agricultural Chemical Society of Japan. (Apr. 4, 1978, Nagoya) Abst. p. 526

36. Interconversion of nucleosides. Takuo SAKAI (Lab. Fermentation Chemistry). in *Microbial Production of Nucleic Acid-Related Substances* (Koichi OGATA, *et al.*, eds.) Kodansha Ltd., Tokyo, and Halsted Press, New York. pp. 226–236 (1976). Also presented in the Japanese language edition (Association of Amino Acid and Nucleic Acid, ed.: *Kakusan Hakko*, Kodansha Ltd., Tokyo, pp. 218–228, 1976)

37. Studies on the Determination Methods for the Halotolerance of *Tetracoccus soyae* and *Bacillus* sp. (Studies on halotolerance of bacteria. Part II). Takuo SAKAI, Yaeko KASAI, Shigeru TAJIMA and Shojiro OMATA (Lab. Fermentation Chemistry). *Hakko Kogaku Zasshi* (*J. Ferment. Technol.*) 54 (11), 766–775 (1976) (*in Japanese with English abstract*).

38. Action of Yeast Lysis Activating Factor (LAF) (Studies of lysis of yeast. Part II). Takuo SAKAI, Morimasa HATA and Haruhiko KAWASAKI (Lab. Fermantation Chemistry). Presented at *1977 Ann. Mtg. Agric. Chem. Soc. Japan* (April 1, 1977, Yokohama) (Abstract, p. 30, *in Japanese*).

LAF accelerated the lysis of living *Pichia mogii* cells with zymolyase$^{T.M.}$ 5000 (Kirin Brewery Co. Ltd.). LAF exhibited an antibitic activity against yeasts and moulds, being attached to near cell membrane and removing respiratory activity in a short time. A similar action was found in a saponin, digitonin, and a mode of action of LAF was discussed in relation to that of digitonin.

39. Action of Sodium Chloride to Cell Surface of halotolerant Bacteria. Takuo SAKAI, Yaeko KASAI and Hirotsugu MISHIMA (Lab. Fermentation Chemistry). Presented at *1977 Ann. Mtg. Agric. Chem. Soc. Japan* (April 2, 1977, Yokohama) (Abstract, p. 226, *in Japanese*)

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Two strains of halotolerant bacteria, *Bacillus* sp. M-9 and *Tetracoccus soyae*, were used. They apparently differ from each other in mode of halotolerance. Chemical composition of cell wall and cell membrane of the bacteria grown in the presence of 15% sodium chloride was compared with those grown devoid of added sodium chloride.

40. Adenosine Deaminase from *Pseudomonas iodinum*. Preliminary Studies of Its Puridication and Some Properties. Takuo SAKAI and Hong-Ki JUN (Lab. Fermentation Chemistry). Presented at *1977 Ann. Mtg. Agric. Chem. Soc. Japan* (April 4, 1977, Yokohama) (Abstract, p. 399, *in Japanese*)

This enzyme was very unstable unless it was dealt with in the presence of 15% ethanol, when its purification first became possible. It was purified about 300-fold by protamine treatment, ammonium sulfate and ethanol fractionations, and DEAE-cellulose and Sephadex G-200 column chromatographies. Some properties of its partially purified preparation were examined.

41. Formation and Accumulation of Methylmercury in Organisms. Nobumasa IMURA, Shoe-Kung PAN, Masanori SHIMIZU, Tyunosin UKITA and Kenzo TONOMURA (Lab. Fermentation Chemistry). *Ecotoxi. Environ. Safety*, 1, 255 (1977)

42. Discussion on Enzymes Concerning Deamination of Adenine-Related Compounds in *Pseudomonas* Bacteria. Takuo SAKAI, Hong-Ki JUN and Kenzo TONOMURA (Lab. Fermentation Chemistry). Presented at *1977 Ann. Mtg. Soc. Ferment. Technol. Japan* (November 12, 1977, Osaka) (Abstract, pp. 129–130, *in Japanese*)

A bacterium which grows on adenosine as the sole carbon and nitrogen sources was isolated and identified as a variety of *Ps. synxantha.* Adenine deaminase in this bacterium and adenosine deaminase in *Ps. iodinum* was purified to the homogenous states. Properties of these enzymes were examined and their rôles in adenosine metabolism were discussed. Distribution of adenine and adenosine deaminases in microorganisms was also described.

43. ATP Synthesis Driven by Light and Induced ΔpH in an Extremely Halophilic Bacterium, R113. Haruhiko KAWASAKI, Kenzo TONOMURA and Shojiro OMATA (Lab. Fermentation Chemistry). Presented at *1977 Ann. Mtg. Soc. Ferment. Technol. Japan* (November 10, 1977, Osaka) (Abstract, p. 148, *in Japanese*)

44. Extraction of Pectin from Citrus Fruit Peel with a Yeast (Studies of utilization of citrus fruit peel. Part I). Takuo SAKAI and Minoru OKUSHIMA (Lab. Fermentation Chemistry). Presented at *1977 Ann. Mtg. Soc. Ferment. Technol. Japan* (November 10, 1977, Osaka) (Abstract, pp. 203–204, *in Japanese*)

A yeast which releases pectin from citrus fruit peel was isolated from rotten vegetables and identified as *Trichosporon penicillatum* SNO-3. The release of pectin was found to be catalized by an enzyme so-called "protopectinase". Enzymic production of pectin was examined, and its assignment to the action of endo-polygalacturonase is discussed.

45. Anion Exchanger-Fixed Formic and Acetic Acid Is Utilized As Carbon Source by Most Yeasts and Bacteria. Takuo SAKAI, Kiyoshi YOKOTA and Tohoru KATSURAGI (Lab. Fermentation Chemistry). Presented at *1977 Ann. Mtg. Soc. Ferment. Technol. Japan* (November 12, 1977, Osaka) (Abstract, p. 329, *in Japanese*)

Cultivation of yeasts and bacteria on formic, acetic or propionic acid fixed on Dowex SBR-P (formerly 21K) as the carbon source was described. Batch, fed-batch and continuous cultures with hollow fibre filter were also designed for this purpose in a like manner for dialysis culture methods with dailysis membranes.

46. Adenosine Deaminase from *Pseudomonas iodinum*. Its Purification and the Properties of the Crystalline Preparation. Hong-Ki JUN and Takuo SAKAI (Lab. Fermentation Chemistry). Presented at *1978 Ann. Mtg. Agric. Chem. Soc. Japan* (April 3, 1978, Nagoya) (Abstract, p. 336, *in Japanese*)

Following the results of preliminary studies, the enzyme was purified to the homogeneous state by column chromatographies on DEAE-cellulose, Sephadex G-200, DEAE-Sephadex A-50, Sephacryl S-200 Superfine and hydroxyapatite, and repeated crystallization from ammonium sulfate solution in the presence of 15% ethanol throughout all the procedures with a yield of 20%. Properties of the

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purified enzyme were described.

47. Protopectinase Activity of *Trichosporon penicillatum* SNO-3 (Studies of utilization of citrus fruit peel. Part II). Takuo SAKAI and Minoru OKUSHIMA (Lab. Fermentation Chemistry). Presented at *1978 Ann. Mtg. Agric. Chem. Soc. Japan* (April 4, 1978, Nagoya) (Abstract, p. 473, *in Japanese*)

Following the results of preliminary studies, the enzyme catalizing the solubilization of pectin was purified to the electrophoretically homogeneous state. This preparation possessed, besides protopectinase activity, pectin liquefying and pectin saccharifying activity, and liquefied pectic acid better than pectin, suggesting that the enzyme is of an endo-polygalacturonase type.

48. Purification and Properties of a Second Enzyme Catalysing the Splitting of Carbon-Mercury Linkages from Mercury Resistant *Pseudomonas* K62. Toshiyuki TEZUKA and Kenzo TONOMURA (Lab. Fermentation Chemistry). *J. Bacteriol.*, 135 (1), 138 (1978)

49. *Alcaligenes* and *Acinetobacter* Strains Capable of Degrading Polychlorinated Biphenyls. Kensuke FURUKAWA, Fumio MATSUMURA and Kenzo TONOMURA (Lab. Fermentation Chemistry). *Agric. Biol. Chem.*, 42 (3), 543 (1978)

50. Effect of Chlorine Substitution on the Biodegradability of Polychlorinated Biphenyls. Kensuke FURUKAWA, Akira KAMIBAYASHI, and Kenzo TONOMURA (Lab. Fermentation Chemistry). *Appl. Environ. Microbiol.*, 35 (2), 223 (1978)

51. Microorganisms Utilizing Light Energy: On Function of Bacteriorhodopsin. Haruhiko KAWASAKI and Kenzo TONOMURA (Lab. Fermentation Chemistry). *Hakko to Kogyo*, 36 (2), 118 (1978) (*in Japanese*)

52. Mercury Pollution and Microorganisms. Kenzo TONOMURA, Masaru YAMADA, Kensuke FURUKAWA and Toshiyuki TEZUKA (Lab. Fermentation Chemistry). *Biseibutsu no Seitai*, 5, 23 (1978) (*in Japanese*)

53. Purification and Crystallization of Adenosine Deaminase in *Pseudomonas iodinum* IFO 3558. Takuo SAKAI and Hong-Ki JUN (Lab. Fermentation Chemistry). *FEBS Lett.* 86 (2), 174–178 (1978)

54. Different Effects of Some Growing conditions on Glycolate Dehydrogenase in Mitochondria and Microbodies in *Euglena gracilis*. Akiho YOKOTA, Yoshihisa NAKANO and Shozaburo KITAOKA (Nutrition Laboratory) Agric. Biol. Chem., **42**, 115–120 (1978)

E. gracilis grown on air with illumination contained glycolate dehydrogenase both in mitochondria and microbodies while that grown on the air containing 5% CO_2 with illumination contained the enzyme only in mitochondria with half an activity. Transfer of cells grown on air with illumination to 5% CO_2 or nitrogen atmosphere or to darkness caused disappearance of the enzyme activity in microbodies remaining it only in mitochondria. *E. gracilis* grown heterotrophically on various carbon sources, except glutamate in single, contained glycolate dehydrogenase only in mitochondria. The enzyme in microbodies is apparently associated with photorespiration while the mitochondrial enzyme with the glycolate metabolism in this organelle, which has functions not related to photorespiration or photosynthesis.

55. Metabolism of Glycolate in Mitochondria of *Euglena gracilis*. Akiho YOKOTA, Yoshihisa NAKANO and Shozaburo KITAOKA (Nutrition Laboratory) Agric. Biol. Chem., **42**, 121–129 (1978)

Difference spectra taken at −196°C were given to confirm that glycolate oxidation by *Englena* mitochondria is linked to the electron transport system with the production of ATP. In feeding experiments with 1-^{14}C-glycolate on *E. gracilis* cells having glycolate dehydrogenase only in mitochondria, greater parts of the taken-up radioactivity were excreted as $^{14}CO_2$ and greater parts of the remaining were distributed to the amino acid and protein fractions, mainly as glycine and serine. Presence of ammonium decreased $^{14}CO_2$ evolution and augmented incorporation and distribution to protein of label. Exhaustion of nitrogen source stopped glycolate uptake. Deficiency of nitrogen source repressed glycolate dehydrogenase most extensively among the enzymes involved in the

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glycolate pathway. The mechanism of regulation of the glycolate metabolism by nitrogen source is discussed.

56. Composition and Variation by Illumination of Carotenoid in Streptomycin-bleached Mutant of *Euglena gracilis z*. Akiho YOKOTA, Kyoko OKAMOTO and Shozaburo KITAOKA (Nutrition Laboratory) Nippon Nogeikagaku Kaishi, **52**, 97–99 (1978)

The permanent bleached (streptomycin) mutant of *Englena gracilis* z contained 0.25 pg/cell of total carotenoid when cultured in a medium with glucose and glutamate as the major carbon sources in the dark for 10 days, while the mutant contained 0.80 pg/cell when cultured with illumination. The dark-grown cells increased the carotenoid content upon exposure to light depending on the light intensity. The dark-grown cells contained antheraxanthin as the major carotenoid constituent; β-carotene, ethinenone and euglenanone were other constituents. Upon illumination of the dark-grown cells, euglenanone was increased 16-fold but the content of antheraxanthin was not changed. In the bleached mutant of *E. gracilis* containing no chloroplasts, euglenanone appears to function to protect cells from damaging action of light.

57. Enzymological Properties of Pantothenate Synthetase from *Escherichia coil* B. Kazutaka MIYATAKE, Yoshihisa NAKANO and Shozaburo KITAOKA (Nutrition Laboratory) J. Nutr. Sci. Vitaminol., **24**, 243–254 (1978)

Following a previous report on physicochemical properties, the enzymological properties of a homogeneously purified preparation of pantothenate synthetase were described. The optimum pH was 10.0 and optimum temperature 30°C. The lyophilized enzyme was very stable on standing at −20°C. K^+ or NH_4^+ and Mg^{2+} or Mn^{2+} were required as activators; other cations examined were inhibitive to various extents and the enzyme required ATP as the energy supplier. Some ω-amino acids exerted strong inhibition, and the enzyme was inhibited by some chelating agents but was not affected by SH compounds and SH inhibitors. Apparent K_m for pantoate was 6.3×10^{-5} M, for β-alanine 1.5×10^{-4} M, and for ATP 1.0×10^{-4} M. According to the method of CLELAND, the enzyme reaction proceeds by a Bi Uni Uni Bi Ping Pong mechanism and a scheme showing the order of binding of substrates and releasing of products is presented.

58. Permiation of Water through the Oil Layer in W/O/W-Type Emulsions. Yoshiko KITA, Sachio MATSUMOTO, and Daizo YONEZAWA (Lab. Food Chem.) J. Chem Soc. Japan 11–14 (1978) (*in Japanese*)

59. Preparation of Water-in-Olive Oil-in–Water Multiple-Phase Emulsions in an Eatable Form. Sachio MATSUMOTO, Yoshiro UEDA, Yoshiko KITA, and Daizo YONEZAWA (Lab. Food Chem.) Agr. Biol. Chem. **42**, 739–743 (1978)

60. Method for Preparing W/O/W-Type Multiple-Phase Emulsions. Sachio MATSUMOTO (Lab. Food Chem.) J. Japan Oil Chem. Soc. **26**, 655–661 (1977) (*in Japanese*)

61. Preparations of W/O/W-Type Multiple-Phase Emulsions and Lipid Vesicle Suspensions. Sachio MATSUMOTO (Lab. Food Chem.) Membrane **2**, 434–443 (1977) (*in Japanese*)

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63. International Symposium on Food Texture and Rheology. Sachio MATSUMOTO (Lab. Food Chem.) J. Japan Soc. Food Sci. Technol. **25**, 354–356 (1978) (*in Japanese*)

64. The Role of Rheology in Food Science. Sachio MATSUMOTO (Lab. Food Chem.) J. Soc. Rheology Japan **6**, 39–44 (1978) (*in Japanese*)

65. Comparison of Glutenin in Various Wheat Flours by Viscometry. Zen-ichiro HAMAUZU and Daizo YONEZAWA (Lab. Food Chem.) Food and Nutrition **28**, 493–496 (1975) (*in Japanese*)

66. Molecular Weight Determination of Gluten Polypeptides by Gel Filtration in 6M Guanidine Hydrochloride. Zen-ichiro HAMAUZU and Daizo YONEZAWA (Lab. Food Chem.) Agr. Biol. Chem.

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42, 1283–1284 (1978)

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68. **The Chemical Structure of a Mannofucogalactan from the Fruit Bodies of Flammulina Velutipes (Fr.) Sing.** Takafumi MUKUMOTO and Haruki YAMAGUCHI (Lab. Food Chem.) Carbohyd. Res. 59, 614–621 (1977)

69. **Recent Aspects of Chemical Methods for Structural Elucidation of Polysaccharides and Complex Carbohydrates.** Haruki YAMAGUCHI (Lab. Food Chem.) Seikagaku 49, 1316–1331 (1977) (*in Japanese*)

70. **Isolation of Amylase Inhibitor-producing Microorganism.** Sawao MURAO, Kunio OHYAMA and Sei OGURA (Lab. Appl. Microbiol.) Agric. Biol. Chem., 41, 919 (1977)

71. **Isolation and Characterization of Oligosaccharides from an Enzymatic Hydrolyzate of Red Yeast Cell Walls.** Moto ARAI and Sawao MURAO (Lab. Appl. Microbiol.) Agric. Biol. Chem., 41, 617 (1977)

72. **Affinity Chromatography of Acid Proteases on SPI Coupled to PAB-Cellulose.** Masami HOSHINO, * Ryuichi MIYAJIMA,* Sawao MURAO and Koji MITSUGI,* (Lab. Appl. Microbiol., *Ajinomoto Co., Inc.) Agric. Biol. Chem., 41, 709 (1977)

73. **Novel Microbial Alkaline Protease Inhibitor, MAPI, Produced by *Streptomyces sp.* No. WT-27.** Sawao MURAO and Takashi WATANABE (Lab. Appl. Microbiol.) Agric. Biol. Chem., 41, 1313 (1977)

74. **The Influence of Temperature and Urea on Intrinsic Fluorescence of *Streptomyces* Subtilisin Inhibitor.** Tomoko KOMIYAMA,* Makoto MIWA,* Sakae SATO and Sawao MURAO (Lab. Appl. Microbiol., *Seikei University) Biochimica et Biophysica Acta, 493, 188 (1977)

75. **Effect of Pepsin-Inhibitor (S-PI) from *Streptomyes naniwaensis* on the Fruit-body Formation of *Favolus arcularius*.** Takao TERASITA,* Matashi KŌNO* and Sawao MURAO. (Lab. Appl. Microbiol. *Kinki University) Nikkinhou, 18, 129 (1977)s,

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82. Effect of Acid Protease Inhibitor (S-PI) and Calcium Ion on the Growth of *Rhodotorula glutinis*. Motoo ARAI, Masami TSUCHIYA and Sawao MURAO (Lab. Appl. Microbiol.) Nippon Nogeikagaku kaishi, **51**, 643 (1977)

83. Purification and Some Properties of Two Alkaline Proteases from *Pencillium lilacinum* No. 2093. Motoo ARAI and Sawao MURAO Agric. Biol. Chem., **41**, 2293 (1977)

84. The State of Tyrosyl and Tryptophyl Residues in a Protein Proteinase Inhibitor (*Streptomyces* Subtilisin Inhibitor) Kunio INOUYE,* Ben'ichiro TONOMURA,* Keitaro HIROMI,* Sakae SATO and Sawao MURAO (Lab. Appl. Microbiol., *Kyoto University) J. Biochem., **82**, 1207 (1977)

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86. New Metallo Proteinase Inhibitor Produced by *Streptomyces nigrescescens* TK-23: S-MPI Sawao MURAO, Kohei ODA, and Toshio KOYAMA. (Lab. Appl. Microbiol.) Agric. Biol. Chem , **42**, 899 (1978)

87. Effect of Protease Inhibitor (S-PI) on the Growth of Various Yeasts. Motoo ARAI, Eiko TSUCHIYA and Sawao MURAO Agric. Biol. Chem., **42**, 1429 (1978)

88. Partial Structure of Glycopeptide Obtained from *Scytalidium lignicolum* Acid Protease A. Sawao MURAO, Naofumi MORITA, Yoshimaru YAMAMOTO and Kohei ODA. (Lab. Appl. Microbiol.) Agric. Biol. Chem., **42**, 1343 (1978)

89. Effect of Pepsin-inhibitor on the Fruit-body Formation of a Few *Basidiomycetes*. T. TERASHTA,* M. KONO,* and S. MURAO (Lab. Appl. Microbiol., *Kinki University) Hakko to kogyo, **56**, 175 (1978)

90. Characterization of Oligosaccharides from an Enzymatic Hydrolyzate of Red Yeast Cell Walls by Lytic Enzyme. Motoo ARAI and Sawao MURAO (Lab. Appl. Microbiol.) Agric. Biol. Chem., **42**, 1651 (1978)

91. Human Plasma α_1-Proteinase Inhibitor: Temporary Inhibition and Multiple Molecular Forms of the Complex with Porcine Trypsin. K. ODA, M. LASKOWSKI, Sr.,* L. F. KRESS* and D. KOWALSKI* (Lab. Appl. Microbiol., *Roswell Park Memorial Institute, New York.) Biochem. Biophys. Res. Commun., **76**, 1062 (1977)

92. Enzymatic Hydrolysis of Natural Lowbiodegradative Polysaccharides Motoo ARAI and Sawao MURAO (Lab. Appl. Microbiol.) Shokuhinkogyo, **4**, 31 (1978)

93. Red Yeast Cell Lysis by Red Yeast Cell Wall Lytic Enzyme and Protease. Motoo ARAI and Sawao MURAO (Lab. Appl. Microbiol.) Agric. Bio. Chem., **42**, 1461 (1978)

94. Physicochemical and Chemical Properties of Red Yeast Cell Wall Lytic Enzyme from *Pencillium lilacinum* Ryohie YAMAMOTO, Motoo ARAI and Sawao MURAO (Lab. Appl. Microbiol.) Agric. Biol. Chem., **42**, 1593 (1978)

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Section IV

1. Development of the Fetal Pituitary-Testicular System Based on Observation of Leydig Cells in Encephalectomized, Hypophysectomized and Control Fetal Rats. Yasunobu EGUCHI, Kazuyoshi ARISHIMA, Tetsuo NASU, mineyuki TODA, Yoshio MORIKAWA and Yoshiyuki HASHIMOTO (Lab. Vet. Anat.) Anat. Rec. **190**, 679–686 (1978)

The time of onset of brain regulation of the pituitary-Leydig cell system in fetal rats was assessed by fetal encephalectomy, which allowed the pituitary to persist *in situ*. The effects of encephalectomy were compared with those of fetal hypophysectomy (surgical decapitation). The parameter for interpretation of these effects was the collective volume of Leydig cells measured by the method of Chalkley ('43). The normal increase in the collective volume of Leydig cells in fetuses encephalectomized on day 17 of gestation and autopsied on day 18 was not retarded, whereas that in decapitated fetuses of the same age was retarded. In all other one-day experimental periods (day 18–19, 19–20, 20–21, and 21–22), the increase in volume was retarded to approximately the same extent in encephalectomized as in decapitated fetuses. The collective volume of Leydig cells continued to increase to some extent without the brain until day 20, after which it ceased to increase. The results suggest that in fetal rats, the brain control of the pituitary-Leydig cell system begins to operate from day 18 of gestation, when the day following overnight mating was designated as day 1 of gestation.

2. Changes in Placental Permeability to Corticosterone and Estradiol-17β toward the End of Gestation in the Rat. Kazuyoshi ARISHIMA, Sanenori NAKAMA, Yoshio MORIKAWA, Yoshiyuki HASHIMOTO and Yasunobu EGUCHI (Labs. Vet. Anat. and Surg.) Experientia **34**, 262–263 (1978)

Radioactivity in the fetal plasma 1 h after maternal injection of ^{14}C-4-corticosterone or ^{14}C-4-estradiol-17β on day 21 of gestation was markedly higher than that 1 h after injection on day 22. Radioactivity in the maternal plasma was not different on these 2 days. The results suggest that the placental permeability to steroids from the mother to the fetus declines toward the end of gestation in the rat.

3. Effect of 3'-Chloro-4-Stilbazole (CS) a Choline Acetyltrassferase Inhibitor on Acetylcholine Metabolism in vitro. Hiroko KOYAMA, haruo KOBAYASHI, Masako NAKAMURA, Kazue TABITA, Kazu TAKENO and Iwao YANAGITY (Lab. Vet. Pharmacol.) Presented at the 84th Meeting of Jap. Vet. Sci. (Oct. 9, 1977, Miyazaki)

The inhibiting effect of CS on various cholinergic parameters *in vitro* was studied to investigate its specificity for inhibiting choline acetyltransferase. It is a potent inhibitor of choline acetyltransferase with an I_{50} of 10^{-5} M and was about 30 times more potent for inhibiting choline acetyltransferase than high affinity choline uptake and more than 100 times than cholinesterase.

It has no effect on carnitine acetyltransferase, spontaneous acetylcholine release from rat brain slices and choline release from rat brain membrane fraction. Thus the compound was selective for choline acetyltransferase.

4. Effect of Methylmercury on Acetylcholine Metabolism in vitro. Haruo KOBAYASHI, Akira YUYAMA and Naonori MATSUSAKA (Iwate Univ.) Kazu TAKENO and Iwao YANAGIYA (Lab. Vet. Pharmacol.) Presented at the 84th Meeting of Jap. Vet. Sci. (Oct. 9, 1977, Miyazaki)

5. Brain Acetylcholine Level and Turnover in Mouse Intoxicated with Methylmercury. Haruo KOBAYASHI, Akira YUYAMA and Naonori MATSUSAKA (Iwate Univ.) Kazu TAKENO and Iwao YANAGIYA (Lab. Vet. Pharmacol.) Presented at the 85th Meeting of Jap. Vet. Sci. (April 4, 1978 Tokyo)

6. Effect of Methylmercury on Mouse Behavior. Haruo KOBAYASHI, Akira YUYAMA and Naonori MATSUSAKA (Iwate Univ.) Kazu TAKENO and Iwao YANAGIYA (Lab. Vet. Pharmacol.) Presented at the 86th Meeting of Jap. Vet. Sci. (Sept. 6, 1978 Gifu)

7. Effects of Loperamide on Acetylcholine and Porstaglandin Release from Isolated Guined pig Ileum. Osamu YAGASAKI, Hiroyuki SUZUKI and Yukinobu SOHJI (Lab. Vet. Pharmacol.) Japan. J. Pharmacol. **28**: (1978)

Loperamide, an effective antidiarrheal agent, was tested to elucidate the site of action which

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underlies its antiperistaltic and further antidiarrheal action. Loperamide, more than 10^{-8} g/ml, inhibited the release of both acetylcholine and prostaglandins during circumferential distension of the intestinal wall in dose dependent manner. The inhibited ACh release by loperamide but not prostanglandins was reversed by naloxone. Although a high concentration of loperamide inhibited the contraction of intestine to ACh, it also inhibited the contraction to nicotine and serotonin at lower concentration which dose not affect the contraction to ACh. It is concluded that loperamide inhibits the peristaltic movement principally by reducing the release of ACh and prostaglandin during circumferntial distension of the intestinal wall *in vitro.*

8. Pathological Studies on Fascioliasis in the Japanese Deer, *Cervus Nippon*. Tamotsu TOMIMURA, Kimiaki HIRAKAWA, Hiroto YOSHIFUSA (Lab. Vet. Patholog.), and Yasunobu NISHITANI (Nara Shika Fund) Presented at the 84th Meeting of Japanese Society of Veterinary Science. (November 11, 1976, Miyazaki)

In the two recent reports of a series of studies on spontaneous fascioliasis in the Japanese deer, *Cervus Nippon,* in Nara Park, the authors have described the results of detailed investigations. These include the prevalence and intensity of the infestation among deer, the snail, *Bakerlymnaea viridis,* that play an important role for the spread of cervine fascioliasis, and also the postmortem cholangiographic changes of the intrahepatic bile ducts of 18 deer affected with varying degrees of hepatic fascioliasis. In the present Work, pathological changes of the liver of diseased animals were described. The results obtained were summarized as follows:

1) Of a total of 21 livers examined, two showed the acute parenchymal phase of infection which was characterized by traumatic hepatitis, and the remaining 19 exhibited the chronic phase of the disease. Depending on the degree of the macroscopical hepatic lesions caused by *Fasciola* sp. of Japanese origin (herinafter referred to as the "Japanese species"), the chronic form was divided into three groups:

Group I (slightly affected livers): It comprises eight livers from which an average of seven adult flukes were recovered. They were characterized by a focal, or segmental ectasias of the intrahepatic duct system, accompanied by varing degrees of thickening of its wall. There was no distortion of the liver although a little amount of fibrosis was detected in the hepatic parenchyma around the diseased bile ducts.

Group II (moderately affected livers): It is composed of five livers from which an average of 15 adult flukes were recovered. They were characterized by rather severe dilatation of one or two area ducts and their branches, accompanied by varying degrees of thickening of their walls. These changes were usually confined to the left lobe of the liver. Local fibrosis was also present in some areas of the left hepatic lobe in two of these livers, but their distortion was not severe.

Group III (severely affected livers): It includes six livers from which an average of 61 adult flukes were recovered, with a maximum of 142 mature flukes. They were characterized by extremely severe dilatation throughout the intrahepatic biliary tree, accompanied by varying degrees of thickening of its wall. The wall of the diseased bile ducts was however pliable and there was no evidence of calcification. In spite of the occurrence of prominent chronic productive cholangitis, distortion of the liver resulted from extensive fibrosis of the left hepatic lobe could not be detected in all but two instances.

2) The histopathological lesions produced by penetrating juvenile liver flukes along passages were evident in the hepatic parenchyma with hemorrhage, exudation of fibrin, necrosis of the liver cells, and inflammatory cell infiltrations in which eosinophils predominated although variable numbers of lymphocytes, plasma cells and macrophages were also seen. The development of considerable dense fibrous tissue reaction which may act as a mechanical barrier to migration of the immature fluke in the liver parenchyma could not be seen. It was considered, therefore, that the free movement of the parasite in the parenchyma had been unimpeded. Furthermore, the formation of the so-called *Fasciola* nodule which is considered one of the typical pathological changes in unsusceptible final hosts, such as white tailed-deer (*Odocoileus virginianus*) and roe deer (*Capreolus capreolus*), was very rare in the case of Japanese deer.

3) One of the most predominant histopathological features of the chronically affected livers was thickening of the walls of the intrahepatic bile ducts, mainly due to excessively marked adenomatous hyperplasia of their lining epitherium which was induced by the long-standing irritation of adult flukes inhabiting them. These markedly thickened walls also showed rather extensive

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superficial necrosis of the mucous membrane with erosions, and an infiltration of variable numbers of eosinophils, small round cells and brilliant cells. The latter is now thought to be derived from smooth muscle cells among some Japanese workers. The mucous membrane of the intrahepatic biliary passages in which many flukes were contained had often been replaced by a granulation tissue. However, calcification in the wall of the affected bile ducts was detected in only two instances where only a small calcified area was seen microscopically in the intrahepatic biliary tree after examining a large number of tissue sections. A considerable replacement of the hepatic parenchyma by fibrous tissue, which may refer in part to the fibrous depositions in the hemorrhagic tracks made by the young flukes but is mainly due to the presence of adult parasites in the bile ducts, could not be detected even in some parts of the liver having extensive lesions in the intrahepatic bile ducts.

4) Although the fibrous perihepatitis which may be due to irritation induced by the immature flukes entering the liver tissues was found in many chronic cases of cervine fascioliasis, there was no evidence of secondary pathological lesions, such as pleuritis and traumatic damage in the lung, pancrease, or lymph nodes caused by the migration of flukes,as having been reported by many authors in cattle and sheep.

5) From the results of the present studies, it is postulated that the Japanese deer, *Cervus nippon*, belongs to the low resistance group like sheep, rabbits, or rats. Considering the fact that the deer is a suitable host species for the "Japanese species" of the liver fluke, preventive measures have to be taken immediately to protect the deer from fascioliasis as well as to avoid the potential hazard of human infection to the disease in the park, where contamination of waterweeds with metacercariae of this fluke is very prevalent.

9. Studies on the Occurrence and Distribution of *Paragonimus Miyazakii* Kamo *et al.*, 1961 in Kinki Districts III. Observations on the Incidence of Encysted Larvae of *P. miyazakii* in *Potamon Dehaani* in the Northern Part of Wakayama Prefecture. Tamotsu TOMIMURA, Hiroto YOSHIFUSA, Shuichi KIN, Hiromu SUGIYAMA (Lab. Vet. Patholog.), Hiroshi NISHIDA, and Masahiro SAKAI (Dept. Parasitolog., Sch. Med., Ehime Univ.) Presented at the 85th Meeting of Japanese Society of Veterinary Science. (April 3, 1978, Tokyo)

During the period from October 1977 to May 1978, the crab, *Potamon dehaani*, which is known as the second intermediate host of the lung fluke, *Paragonimum miyazakii*, was collected at 16 places in the northern part of Wakayama Prefecture. One thousand four hundred and ninety-three crabs were examined for the metacercariae of *P. miyazakii*. A total of 2424 encysted larvae of this lung fluke were obtained from 440 of 1108 crabs captured at the eight places. The number of metacercariae per crab host varied from 1 to 24, with an average of 5.51. No larval lung fluke infestation was found in 385 individuals from the remaining eight spots. On the basis of their morphological features, all the larvae were identified as *P. miyazakii* Kamo *et al.*, 1961. Moreover, the adult lung flukes and their eggs recovered from the cats fed on the metacercariae in question, were so identified, also. By the present paper, the northwestern part of Wakayama Prefecture must be added to the list of known prevalent areas of this lung fluke in the Kinki districts of Japan.

10. Pathological Studies on Canine Herpesvirus Infection. I. Spontaneous Cases of Newborn Puppies. Takao KOTANI, Hiroshi MOCHIZUKI (Lab. Vet. Patholog.), Syusaku NODA (Lab. Vet. Int. Med.) and Minoru MAKINO (Practicing Veterinarian) Presented at the 84th Meeting of Jap. Soc. Vet. Sci. (November 11, 1977, Miyazaki)

Five purebred Pug puppies of a litter, apparently normal at birth, suddenly became ill and died at various intervals during the 12th and 16th day of life. Two of these puppies were pathologically diagnosed as a canine herpesvirus infection.

The cardinal clinical signs were painful whining, laboured breathing and nasal discharge.

The main pathological lesions were focal necrosis and hemorrhage in the lungs, kidneys, liver, nasal mucosa, small intestine, spleen, adrenal glands, pancreas and cerebellum. Acidophilic intranuclear inclusions were observed in the epithelial cells, interstitial cells and vascular endothelial cells in the necrotic foci.

By electron microscopical examination, viral particles were detected in both the nuclei and the cytoplasm of the infected cells. Naked capsids measured 90–100nm in diameter, and enveloped forms were about 150nm across. The nucleoids in some of the virions showed ring, cross and star forms.

11. Pathological Studies on Canine Herpesvirus Infection. II. Lesions of Puppies Infected with Intraperitoneal and Subcutaneous Inoculation. Takao KOTANI, Yoshiharu ODAGIRI and Hiroshi

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MOCHIZUKI (Lab. Vet. Patholog.) Presented at the 85th Meeting of Jap. Soc. Vet. Sci. (April 4, 1978, Tokyo)

Six mongrel puppies (one day old) of a litter were inoculated intraperitoneally (3 puppies) and subcutaneously (3 puppies) with supernate of homogenated lung, liver and kidney of the naturally occurring case of canine herpesvirus infection.

All the dogs showed constant signs and lesions which varied only slightly in intensity. Serous nasal discharge was observed in all puppies at 2 to 3 days after inoculation. They were dying between 5th and 6th day postinoculation. The prominent lesions were focal necrosis with intranuclear inclusions and hemorrhage in the lungs, intestine, stomach, liver, kidneys, spleen, adrenal glands, pancreas, nasal mucosa, trachea, heart muscle, thymus, bone marrow, leptomeninx, cerebellum, cerebrum and retinas. Moreover, numerous viral particles of herpesvirus were observed by electron microscopy in the nuclei and cytoplasm of infected cells in many organs.

12. Paradoxical Embolism with *Dirofilaria immitis* in Dogs. Takao KOTANI, Tamotsu TOMIMURA, Tetsuro SAKAMOTO and Hiroshi MOCHIZUKI (Lab. Vet. Patholog.) Presented at the 86th Meeting of Jap. Soc. Vet. Sci. (September 6, 1978, Gifu)

Out of a total of 1,521 canine cases autopsied routinely in our laboratory, 34 were diagnosed as paradoxical embolism with *Dirofilaria immitis*, in which vestigial structures, such as the patent foramen ovale (PFO) may cause critical infarction in the hind legs and viscera. Probable pathways of the worm into the systemic arterial circulation were PFO in most cases (32/34) and atrial septal defect and ventricular septal defect each in a single case.

13. Immunological Aspect of Clostridium perfringens Food Poisoning. Reidar SKJELKVÅLE and Takashi UEMURA (Lab. Vet. Publ. Health) Presented at the 5th International Congress of Food Science and Technology (Sep. 19, 1978, Kyoto)

To understand the mode of action of the enterotoxin from *Clostridium perfringens*, more information is needed to clarify the interactions between toxin and the intestinal tract. Results in a series of our experiments were summarized as follows:

(1) After an outbreak of food poisoning as well as oral administration of enterotoxin to experimental animals, anti-enterotoxin titer in serum was increased. (2) Immunization by oral route in chickens have some protective effect on fluid accumulation induced by enterotoxin in the ligated intestinal loops, but passively administered anti-enterotoxin did not reveal the effect. (3) Epithelial cells from rabbit ileum bound 30 times more ^{125}I-labelled enterotoxin per mg than ^{125}I-labelled bovine serum albumin, and a small amount of the labelled enterotoxin passed the intestinal border to the blood stream.

14. Experimental Diarrhoea in Human Volunteers Following Oral Administration of Clostridium perfringens Enterotoxin. Reidar SKJELKVÅLE and Takashi UEMURA (Lab. Vet. Publ. Health) J. Appl. Bacteriol., 43, 281–286 (1977)

15. Incidence of Enterotoxigenic Clostridium perfringens in Healthy Humans in Relation to the Enhancement of Enterotoxin Production by Heat Treatment. Takashi UEMURA (Lab. Vet. Publ. Health) J. Appl. Bacteriol., 44, 411–419 (1978)

16. Sporulation and Enterotoxin Production by Clostridium perfringens on Solidified Sporulation Media. Takashi UEMURA (Lab. Vet. Pub. Health) J. Food Hyg. Soc., 19, 462–467 (1978)

17. Intestinal Absorption of Botulinum Toxins of Different Molecular Sizes in Rats. Shunji SUGII, Iwao OHISHI, and Genji SAKAGUCHI (Lab. Vet. Publ. Health) Infect. Immun., 17: 491–496 (1977)

During a period of 10 to 12 h after injection of type B 16S (L) toxin into the ligated duodenum of rats, 0.01 to 0.1% of the total toxicity administered was found in the lymph drawn by cannulation of the thoracic duct. The recovery was 50 to 100 times higher than that of the rat given type B 12S (M) or 7S (S) toxin. During the same period, 0.6 to 1.5% of the specific antigens were recovered, regardless of the molecular size of the toxin that had been adminstered. In lymph of the B–L or B–M toxin recipient, the toxic and non-toxic components were detected in comparable quantities, indicating that the undissociated progenitor toxin molecules is abosrbed through the intestinal wall. Although the toxic component had lost its toxic activity, the two components of B–M toxin

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appearing in lumph reassembled to reconstruct the 12S molecule, whereas those of B–L toxin did not, although the toxic component was still active. Type B–L, B–M, and B–S toxins showed similar stabilities to in vitro exposure to rat lymph (pH 8.2), but B–L toxin showed a considerably higher stability to intestinal juice (pH 7.0) than did B–M toxin. Thus, the toxicity of lymph of rats administered botulinum toxin intraduodenally depends not upon the rate of absorption, but largely upon the stability in the intestine.

18. Development of Antitoxin with Each of Two Complementary Fragments of Clostridium Botulinum Type B Derivative Toxin. Shunji KOZAKI, Shuichi MIYAZAKI, and Genji SAKAGUCHI (Lab. Vet. Publ. Health) Infect. Immun., 18: 761–766 (1977)

Two fragments with molecular weight of 111,000 (fragment I) and 59,000 (fragment II) were separated from each other by gel filtration of dithiothreitol and urea-treated, trypsinized derivative toxin (molecular weight, 170,000) of the proteolytic Okra strain of *Clostridium botulinum* type B on Sephadex G-200 (superfine) with a buffer containing dithiothreitol and urea. Upon removal of dithiothreitol and urea by dialysis, the two fragments reassembled to reconstruct the derivative toxin molecule. Both fragments were immunogenic, and both anti-fragments neutralized type B toxin. The neutralizing activities of both anti-fragment I and anti-fragment II were lower than that of the anti-derivative toxin, suggesting that the molecular integrity of derivative toxin is essential for sufficient production of the neutralizing antibody. The immunological difference found between type B toxin from a proteolytic strain and that from a nonproteolytic strain was ascribed to the antigenic difference of fragment I.

19. Purification of Staphylococcal Enterotoxin E and Antigenic Similarity between Enterotoxin A and E. Kunihiro SHINAGAWA, Nobuharu KUNITA (Osaka Prefec. Inst. Publ. Health) and Genji SAKAGUCHI (Lab. Vet. Publ. Health) Japan. J. Bacteriol., 32: 829–836 (1977)

Staphylococcal food poisoning is caused by ingestion of enterotoxin. Diagnosis of staphylococcal food poisoning should, therefore, depend upon detection of enterotoxin in the incriminated foodstuff or in cultures of staphylococci isolated from it. For immunological detection of enterotoxin, specific anti-enterotoxin sera are necessary.

Bergdoll et al. (1971) stated that enterotoxin E was neutralized with anti-enterotoxin A serum and formed a precipitin line against this serum. Terayama et al.(1974) reported that enterotoxin E did not react to antienterotoxin A serum.

The authors purified enterotoxin E by essentially the same method as those utilized to purify enterotoxin A, B, and C.

Purified enterotoxin E (120 μg/ml) formed a distinct precipitin line against anti-enterotoxin A serum. This line fused with that of enterotoxin E appearing against homologons serum. At a low consentration of the toxin (20 μg/ml), the precipitin line against anti-enterotoxin A serum and that against anti-enterotoxin E serum coalesced with each other. In contrast, enterotoxin A at 120 μg/ml, reacting to anti-enterotoxin E serum, formed a precipitin line which was fused with that against anti-enterotoxin A serum. When 20 μg/ml of enterotoxin A was used, no precipitin line was formed against anti-enterotoxin E serum.

20. Exprimental Botulism in Chickens: The Secum as the Site of Production and Abosrption of Botulinum Toxin. Shuichi MIYAZAKI and Genji SAKAGUCHI (Lab. Vet. Publ. Health) Japan. J. Med. Sci. Biol., 31: 1–15 (1977)

Highly purified preparations of *Clostridium botulimum* toxins were administered to chickens by various routes. Chickens were highly susceptible to type A toxin, but relatively resistant to toxins of other types. Type C toxin (12S) at a dose of 1×10^7 mouse ip LD_{50} failed to kill the chicken by the oral route. Oral administration of 10 or more of type A, C, or D spores killed normal chickens, whereas cecoligated chickens were insusceptible to oral adminstration of 10^6 spores. These results show that the site of production and abosrption of botulinum toxin in chickens is the cecum.

Oral adminstration of spores of a type C strain cured of its prophages and producing the C2 factor only also killed normal chickens. Chickens are more susceptible to the C2 factor than to the Cl toxin. The C2 factor may play the crucial role in chicken deaths from toxico-infection with type C organisms.

The optimum temperature for the growth of *C. botulinum* types C and D was found to be 40–42C. Type C and D toxins were significantly more stable than type A toxin in the cecum contents with pH above 7. These characteristics and the high density of distribution of type C spores in the

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environment may explain the prevalence of type C botulism among broiler chickens.

21. Botulogenic Properties of Vegetables with Special Reference to the Molecular Size of the Toxin in Them. Shunji SUGII and Genji SAKAGUCHI (Lab. Vet. Publ. Health) J. Food Safety, 1: 53–56 (1977)

The molecular sizes of *Clostridium botulinum* type A, B, E, and F toxins produced in string beans, mushrooms, tuna fish and pork were determined to provide an explanation for the high botulogenic properties of vegetables. Type A and B organisms produced the orally more toxic 16S and 19S molecular-sized toxins in vegetables, whereas they produced the orally less toxic 12S and only rerely some 16S toxin in tuna fish and pork. Type E and F organisms produced only 12S toxin in any food or culture medium, but addition of glucose seemd essential for appreciable toxin production. It appeared that the molecular sizes of type A and B toxins transform depending upon the contents of iron or manganese salts in foods.

22. Acid Precipitation of Clostridium Botulinum Type C and D Toxins from Whole Culture by Addition of Ribonucleic Acid as a Precipitation aid. Masakazu IWASAKI, and Genji SAKAGUCHI (Lab. Vet. Publ. Health) Infect. Immun., 19: 749–751 (1978)

The ratios of ribonucleic acid to protein contents of *Clostridium botulimum* type C, D, and E cultures were lower than those of type A, B, and F cultures. Addition of ribonucleic acid at 0.4 mg/ml to culture satisfactorily aided acid precipitation of type C and D toxins, but not that of type E toxin.

23. Botulism of New Type: Intraintestinal Toxin Production in Infants and Broiler Chickens (A review) Genji SAKAGUCHI (Lab. Vet. Publ. Health) Japan. J. Bacteriol., 33: 673–680 (1978)

Botulisms in infants and broiler chickens, that are considered to involve a hetherto unknown pathogenesis, have been reviewed and explained. From the results of experimental chicken infection with botulinum spores obtained in this laboratory, pathogenesis of infant botulism has been discussed with special reference to the site of production and absorption of the toxin in the alimentary tract.

24. Sites of Production of Sex Steroids: Secretion of Steroids from X-Irradiated and Polycystic Ovaries of Rats. Tsutomu SAWADA and Shigetaka ICHIKAWA (Lab. of Animal Reproduction) Endocrinology **102**, 1436–1444 (1978)

Ovaries of immature rats and PMS-induced pregnant rats were unilaterally x-irradiated. Ten days later, the concentrations of pregnane compounds in the ovarian venous plasma were measured. LH (2 μg) was injected iv 30 min before bleeding. A comparison of steroid levels in the ovarian venous effluent of rats with and without destruction of selected tissue components by irradiation of the ovaries suggests that the follicles contribute to the secretion of 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one in the presence of interstitial gland tissue Because it is known that follicular tissue is involved in the production of estrogens, we studied the interrelationship between the secretion of the two progesterone metabolites and estrogens in follicular polycystic ovaries of androgen-sterlized rats. Normal ovaries of diestrus-2 rats were used as controls for the polycystic ovaries. The injection of LH greatly increased the secretion of 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one within 1 h in normal ovaries, but the response of polycystic ovaries was low, suggesting low 5α-reductase activity in the cystic ovary. The polycystic ovaries exhibited a marked increase in the secretion of estrogens in response to LH, whereas normal ovaries showed no significant change. These results suggest that low 5α-reductase activity may be causally related to the high level of estrogen secretion in polycystic ovaries of androgen-sterilized rats.

25. Respiratory Control of the Mitochondria from Brown Adipose Tissue. Tsukasa SUGANO, Masakazu SHIMADA, Tadashi OTA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Peported at the 79th Meeting of Japanese Society of Veterinary Science (April 8, 1975, Tokyo) (*in Japanese*)

26. Glucose Metabolism in Perfused Liver of Cold Exposure Rat. Kohei SUDA, Tsukasa SUGANO, Masakazu SHIMADA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 81th Meeting of Japanese Society of Veterinary Science (April 7, 1976, Tokyo) (*in Japanese*)

27. Respiratory Control of the Mitochondria from Brown Adipose Tissue: Effect of Cold Exposure (I). Tsukasa SUGANO, Masakazu SHIMADA, Tadashi OTA and Hiroshi TATSUMI (Lab.

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Vet. Physiol and Feeding) Reported at the 81th Meeting of Japanese Society of Veterinary Science (April 7, 1976, Tokyo) (*in Japanese*)

28. **Cold Exposure and Catecholamine.** Kayoko HAYASHI, Tsukasa SUGANO, Masakazu SHIMADA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 81th Meeting of Japanese Society of Veterinary Science (April 7, 1976, Tokyo) (*in Japanese*)

29. **Effect of Body Water Contents on the Energy Metabolism of the Rabbit.** Daiji YOSHIWARA, Masakazu SHIMADA, Tadashi OTA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 81th Meeting of Japanese Society of Veterinary Science (April 7, 1976, Tokyo) (*in Japanese*)

30. **Respiratory Control of the Mitochondria from Brown Adipose Tissue: Effect of Cold Exposure (II).** Tsukasa SUGANO, Masakazu SHIMADA, Tadashi OTA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 82th Meeting of Japanese Society of Veterinary Science (October 10, 1976, Iwate) (*in Japanese*)

31. **A Study of Hindlimb Muscle Perfusion of Rat.** Masakazu SHIOTA, Tsukasa SUGANO, Masakazu SHIMADA, Tadashii OTA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 83th Meeting of Japanes Society of Veterinary Science (April 4, 1977, Kanagawa) (*in Japanese*)

32. **Effects of Catecholamine on Carbohydrate Metabolism in Perfused Rat liver.** Kohei SUDA, Tsukasa SUGUNO, Masakazu SHIMADA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 83th Meeting of Japanese Society of Veterinary Science (April 4, 1977, Kanagawa) (*in Japanese*)

33. **Glucose Metabolism in Perfused Rat Liver: Influence of Catecholamines on Gluconeogenesis.** Masakazu SHIOTA, Akihito TARUI, Tsukasa SUGANO, Masakazu SHIMADA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 84th Meeting of Japanese Society of Veterinary Science (November 9, 1977, Miyazaki) (*in Japanese*)

34. **Effect of Glucocorticoid on Rats Exposed to Cold.** Akihoto TARUI, Tsukasa SUGANO, Masakazu SHIMADA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 85th Meeting of Japanese Society of Veterinary Science (April 4, 1978, Tokyo) (*in Japanese*)

35. **On Movement of Intestinal Content into Chicken Cecum.** Takahisa ENDO and Masakazu SHIMADA (Lab. Vet. Physiol. and Feeding) Reported at the 85th Meeting of Japanese Society of Veterinary Science (April 4, 1978, Tokyo) (*in Japanese*)

36. **Effects of Hormones on Glucose Uptake in Perfused Skeletal Muscle.** Masakazu SHIOTA, Tsukasa SUGANO, Masakazu SHIMADA and Hiroshi TATSUMI (Lab. Vet. Physiol. and Feeding) Reported at the 85th Meeting of Japanese Society of Veterinary Science (April 5, 1978, Tokyo) (*in Japanese*)

37. **On Movement of Intestinal Content into Chicken Cecum. – Involvement of Intestinal Nerve–.** Takahisa ENDO and Masakazu SHIMADA (Lab. Vet. Physiol. and Feeding) Reported at the 86th Meeting of Japanese Society of Veterinary Science (September 6, 1978, Gifu) (*in Japanese*)

38. **Biochemical and Ultrastructural Evaluation of Isolated Rat Liver Systems Perfused with a Hemoglobin-Free Medium.** Tsukasa SUGANO, Kohei SUDA, Masakazu SHIMADA (Lab. Vet. Physiolog. and Feeding) and Nozomu OSHINO (Develop. and Res. Dept. of Nihon Schering K.K.) J. Biochem. 83, 995–1007 (1978)

39. **Changes in Excretion of Catecholamines and Metabolites during Acclimation to Cold.** Kayoko HAYASHI, Tsukasa SUGANO, Masakazu SHIMADA, and Hiroshi TATSUMI (Lab. Vet. Physiolog. and Feeding) J. Physiol. Soc. Japan **40**, 39–41 (1978)

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40. Relation between Environmental Temperature and Effect of Local Skin Cooling of the Goat on the Physiological Function. Tadashi OTA and Hiroshi TATSUMI (Lab. Vet. Physiology and Feeding) Reported at the 68th Meeting of Japanese japanese Soc. of Zootechnical Sci. (April 4, 1978)

The effect of local skin cooling of the goat on the physiological function in hot environment was reported previously. The present experiments are concerned with the influence of local skin cooling in optimum and cold temperature. Using two goats, physiological function of the animal was observed at three levels of controlled ambient temperature; 35°C, 20°C and 5°C. Both sides of withers of the goats were locally cooled at −7.5°C during 2.5 hrs with the electric method.

1. Rised rectal temperature of the goat in hot room fell to its normal level by local cooling. In optimum temperature, the rectal temperature fell slightly in rest state and it fell more and more after local cooling. In cold room, the rectal temperature rised slightly due to the increment of heat production by local cooling, although a temporaly fall was observed in the process.

2. The heart rate of the goat in hot room increased continuously until it reached to a high constant level. In optimum temperature, however, the heart rate showed no remarkable change and increased only after local cooling. In cold room, the heart rate increased rapidly. After local cooling, the rate of increase got slow and the heart rate began to decrease to normal level after 1 hr.

3. Increased respiration rate in hot room recovered to its normal level by local cooling. In optimum temperature, however, no change of the respiration rate was observed. In cold room, the respiration rate increased gradually and it increased somewhat quickly after local cooling.

4. The pulmonary ventilation of the goat decreased after local cooling in hot room. On the other hand, it increased by local cooling in optimum temperature and in cold room.

5. Total O_2 consumption and total CO_2 expiration changed in the same courses as those of pulmonary ventilation in each three temperature levels.

Thus, local cooling showed certain reactions as cold stress in optimum temperature and cold environment, but it diminished the reaction in hot environment. It is concluded that local skin cooling of the goat has good effect to prevent the thermal stress only in hot environment.

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