HIGHLIGHTS OF PHARMACOLOGY IN JAPAN[1,2]

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Since 1952, studies on the factors regulating ATP contraction of muscle models, especially of glycerinated muscle fibers, have been energetically conducted at the Department of Pharmacology, University of Tokyo for the primary purpose of inquiring into the mechanism of so-called "excitation-contraction coupling." In 1955, Kumagai, Ebashi & Takeda (1), starting with the crude relaxing factor of Fujita (2), were able to identify the essential relaxing factor as the sediment fraction obtained by centrifuging at 18,000 g for one hour. Emphasis (3, 4, 5) has been laid on the identity of the fraction with the preparation of Kielley-Meyerhof's ATPase. Further development of this work was recently done by Ebashi & Lipmann (6) at the Rockefeller Institute. According to their findings, the fraction of essential relaxing factor identified as a vesicular component (that is, the sarcoplasmic reticulum) can combine calcium in an ATP-linked reaction stronger than ethylenediaminetetraacetic acid. This calcium binding is considered to represent a mechanism for accumulating inorganic ions in living organisms. On the other hand, Ebashi (7) has revealed the essential role of a minute amount of calcium in the mechanism of actomyosin syneresis with ATP, which is in good accord with the results of Weber (8) derived from the studies on actomyosin ATPase.

On the basis of these findings, Ebashi has postulated that the relaxing action of the sarcoplasmic reticulum is due to its extraordinarily strong calcium-binding activity, i.e., calcium located in the vesicular structure is the key substance for the excitation-contraction coupling.

Since 1940 a series of researches have been carried out in Japan on the toxicity of the yellowed rice polluted by *Penicillium toxicarium* [Miyake, Naito & Tsunoda (9)]. In 1948 yellowed grains were also found in rice imported from Spain, as a result of pollution by *Penicillium islandicum* Sopp. Kobayashi, together with Uraguchi *et al.* at the Department of Pharmacology, University of Tokyo, began to investigate the toxicity of these polluted rice grains and to identify the toxins involved (10 to 13). Quite recently Kobayashi, Uraguchi, and associates (13 to 16) have successfully

[1] The survey of the literature pertaining to this review was concluded in April, 1960.

[2] Abbreviations used in this chapter include: ATPase (Adenosine triphosphatase).

isolated two kinds of active principles from the toxins produced by *P. islandicum* Sopp. One is a chlorine-containing peptide, and the other a yellow pigment named luteoskyrin; both were hitherto unknown. Repeated administration to mice and rats has shown that these toxins may produce cirrhosis of the liver. In addition to these findings, Kobayashi, Uraguchi *et al.,* in collaboration with Miyake, Saito, and their collaborators at the Department of Pathology of the University of Tokyo, have also demonstrated in rats by long-term feeding (up to 622 days) that the yellowed rice inoculated by *P. islandicum* Sopp is capable of inducing primary hepatic carcinoma. These findings may throw light on the problem of high incidence of hepatic carcinoma in Orientals.

In 1938 Okada & Mimura (17) devised a method of vital staining of hard tissues. In this method a small quantity of lead acetate is injected into an animal, and the lead deposited in hard tissues is histochemically shown as a distinct fine line. By this means, it became possible to mark the lapse of time in hard tissues, such as teeth or bone, in a living body under desired experimental conditions. Numerous results have already been published (18). For vital staining, a solution of lead acetate is injected into the ear vein of a rabbit; the animal is sacrificed after an appropriate lapse of time, and its teeth are removed and fixed in formaldehyde solution. The teeth are decalcified in 0.2 *N* hydrochloric acid (acetic or phosphoric acid may be used), saturated with hydrogen sulfide, and frozen sections are prepared in order to detect lead lines on dentine. In the decalcified section of the dentine, there is a striated pattern around the pulp cavity, like annual rings in wood, that is stained by hematoxylin. The layers stained by this dye are formed during the night, and those not stained are formed during the day. It was shown that incisor dentine of the rabbit is being formed incessantly at the rate of about 25 μ per 24 hours and calcium metabolism *in vivo* is thus recorded as a wave pattern in dentine. Okada & Asoda have also discussed the authenticity of hematoxylin staining of decalcificated dentine, which is taken as the indication of the degree of calcium deposition in the dentine method. They (19) have shown that the calcium deposition in dentine is the function of blood level of calcium (especially of calcium ion) and of inorganic phosphorous, and that dentine is a far more sensitive indicator of calcium deposition than bone.

Nakao *et al.* at the Department of Pharmacology, Tokyo Jikei-Kai Medical College have been engaging in the studies of biotransformation of steroids in living bodies and tissues. [In this review only one (20) of their recent contributions will be cited.] Their studies are concerned with the identification of the steroids in the human sweat secreted under high environmental temperatures. The experiments were conducted on healthy human subjects of 20 to 30 years of age in a perspiration chamber, and the sweat was collected by the method devised by Takamatsu (21). The room temperature was kept at 38° to 40°C. for four hours and 42° to 45°C. for two hours. The humidity was kept at 70 to 90 per cent. They obtained four

steroid fractions by paper chromatography. Two of them were identified, whereas the quantities of the remaining compounds were too small for complete identification. The toluene-propylene glycol system of Zaffaroni, benzene-methanol-water system of Bush, and ligroine-*tert.* butanol system of Eberlin and Bongiovanni were used for development. One compound was proved to be Δ^4-pregene-11β, 17α-, 20-triol-3-one. This compound was shown to have an antidiuretic action in water-loaded rats. The second steroid was identified as a compound having a Δ^4-3-keto group and an α-ketol group at C17. Although they have not yet been able to confirm the presence of aldosterone in human sweat, they presume that this second compound might be a metabolite closely related to aldosterone.

In 1938 at Osaka University's Department of Pharmacology, Imaizumi (22) expressed the view that some oxidation and methylation process on the side chain of DOPA might occur in the adrenal gland, because he had demonstrated the presence of an epinephrine-like pressor substance in adrenal tissue after the decarboxylation of DOPA in the liver. After further studies, Imaizumi now postulates that besides the main pathway of epinephrine biosynthesis from DOPA by way of hydroxytryptamine and norepinephrine there is another possible pathway (written as tyramine → hydroxytyramine → norepinephrine → epinephrine) because his co-worker Koyabu, recently found a copper-containing enzyme, which is capable of oxidizing tyramine to hydroxytyramine, in rabbit serum and liver.

It is of interest to note here Imaizumi's turn-over theory (23) concerning the mechanism of epinephrine sensitization by amino acid in the blood vessels. According to Imaizumi's theory, epinephrine is first oxidized to epinephrinequinone which in turn acts as an acceptor of hydrogen derived from the oxidation of amino acids. In this way amino acids accelerate the turn-over rate of epinephrine ⇌ epinephrinequinone. He also postulates the same mechanism for ascorbic acid which acts quite similarly to amino acids in this respect.

The brilliant work done by Okamoto at the Department of Pharmacology and Research Institute of Tuberculosis, Kanazawa University should not be overlooked. In 1939 it was found by Okamoto (24) that hemolysin (streptolysin S) production of hemolytic streptococci is markedly enhanced by the addition of yeast nucleic acid to the culture medium. This striking effect, designated as "RNA-effect," was confirmed and extended by many other research workers [Bernheimer (25); Egami (26); and others]. Among findings connected with the RNA-effect, those to be mentioned here are: (*a*) The RNA-effect appears to be specific for beta-hemolytic streptococci and is not shared by any other bacterial species so far examined, with the exception that tobacco mosaic virus ribonucleic acid, ribonucleic acid from certain other sources such as mammalian liver, wheat, and bacteria, and Ochoa's biosynthetic poly-ribonucleotide of adenine, guanine, uracil, cytosine (1 : 1 : 1 : 1) type (27) were shown to be effective in streptolysin S formation. It was thus supposed that hemolytic streptococci and ribonucleic

acid are in close metabolic relationship with each other. (*b*) 2,2′-Dihydroxy-azo-benzene and its tetrabromo derivative were found to be highly inhibitory against the formation of streptolysin S—a finding which suggests that the compounds might also act as potent inhibitors of nucleic acid metabolism in other living cells. (*c*) If well-washed living hemolytic streptococci are made to act *in vitro* on Ehrlich ascites carcinoma or Yoshida ascites sarcoma cells or even leucocytes, an appreciable amount of streptolysin S is produced. In this case, the toxin may be regarded as being produced at the expense of ribonucleic acid contained in the tumor cells or leucocytes.

With these facts in mind, Okomata *et al.* are now conducting two kinds of anticancer studies (28). The first type of experiments are with hemolytic streptococci. Evidence has been provided by Koshimura *et al.* to show that living hemolytic streptococci, regardless of their virulence for mice, have a characteristic ability to affect tumor cells *in vitro* and *in vivo*. Since streptolysin S alone was found to be entirely ineffective in this respect, it was inferred that the anticancer effect shown by the cocci is mainly attributable to their ability to affect the ribonucleic acid in tumor cells. Recently, these workers have successfully prepared cell-free extracts of the cocci, which may exhibit either RNA-effect (streptolysin S formation) or anticancer activity. It is of interest to recall here earlier reports concerning the observations that complete or partial regression of malignant tumors was noticed during and after intercurrent streptococcal infections (Busch, 1886; Fehleisen, 1882-83; Bruns, 1888; Coley, 1891; etc.). The second group of anticancer studies are those experiments on the carcinostatic activity of 2,2′-dihydroxy-azo-benzene derivatives. A number of compounds in the 2,2′-dihydroxyazo-benzene series were synthesized by Hirata; among them, Azo-106 bis(2-hydroxy-3,5-dibromophenylazo)-n-propylphloroglucinol, a potent inhibitor of RNA-effect, was shown to be especially effective against Ehrlich ascites carcinoma, Sarcoma 180, and Yoshida ascites sarcoma.

The working team of Kyoto University has made a distinct contribution to the neuropharmacological approach to the study of the site of action of analgesics. The action of morphine and its congeners on the various afferent pathways of pain was studied by Fujita *et al.* (29, 30) using electrophysiological techniques. In the cat, these investigators showed that morphine (6 mg./kg.) and meperidine (12 mg./kg.) blocked splanchnic afferents in the spinothalamic tract and vagal afferents in the medulla oblongata, but did not block afferents from the sciatic nerve. The same doses of morphine and meperidine abolished the second component of cortical potentials evoked by local cortical stimulation, recruiting responses evoked by stimulation of thalamic intralaminar nuclei and augmenting responses evoked by repetitive stimulation of the medical lemniscus. Yamamoto (31) reported that the site of action of Ohton, 3-dimethylamino-1, 1-di-(2′-thienyl)-1-butene, a potent morphine-like analgesic, was the same as that of morphine. Matsumura *et al.* (32) observed the synergism between morphine and methamphetamine in depressing the cortical potentials evoked by splanchnic stimulation, as well

as recruiting responses and augmenting responses in the cat. In the cat and rabbit, Fujita *et al.* (*29*) and Yasuhara (33) reported that chloral hydrate (100 mg./kg.), urethane (400 mg./kg.), and the inhalation of ether abolished cortical potentials evoked by sciatic, splanchnic, and vagal stimulation, and that barbiturates did not abolish these potentials. Takagi *et al.* (34) and Ogiu *et al.* (35) have studied the action of morphine and meperidine on the reflex arcs of spinal cord using the intact cat, thalamic, midbrain, high-spinal, and low-spinal cat. In all of the preparations except the low-spinal cat, these investigators found that morphine (7 mg./kg.) depressed both the monosynaptic and multisynaptic reflex discharges evoked by single shocks applied to the ipsilateral sciatic nerve. In the low-spinal cat (transected at T_1–T_2 or L_2–L_3 levels) no inhibitory effect of morphine was observed, even after 14 mg./kg. of morphine. In addition, those investigators found that in cats with lesions in the brain-stem reticular inhibitory area, morphine enhanced both types of reflexes; whereas in cats with lesions in the reticular facilitatory area, morphine abolished the polysynaptic reflex discharges and reduced the monosynaptic reflex discharges. These findings suggest that morphine affects spinal reflex indirectly through stimulation of facilitatory and inhibitory centers in the brainstem, with inhibitory effects usually predominating. Similar results were obtained with meperidine.

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