Antidiuretic Action of Enteramine

Previous investigations[1] have shown that acetone extracts of posterior salivary glands of *Octopus vulgaris* and *Eledone* (*E. moschata* and *E. Aldrovandi*) contain a principle which markedly reduces the diuresis of hydrated rats.

Later and more extensive researches, which will be reported in detail in future papers, have confirmed these first observations, allowing us to individualize and characterize the antidiuretic principle more accurately.

This is now to be identified, with reasonable certainty, as the enteramine-like substance which for several years has been known to exist in the posterior salivary glands of the *Octopoda* mentioned above. In fact:

(1) The field of distribution of the antidiuretic principle strikingly covers that of enteramine (or enteramine-like substances), both in the different animal species and in the different organs of an individual animal. Indeed, not only extracts of salivary glands of *Octopus vulgaris* and *Eledone* possess antidiuretic activity, but also extracts of the hypobranchial body of *Murex trunculus* and *Murex brandaris*, as well as, although to a lesser extent, extracts from ox spleen, gastric mucosa of rabbits and dogs, and duodenal mucosa of oxen and dogs. Extracts of hepato-pancreas, kidney, gill, intestine, ovary, testicle, tentacle muscle and hemolymph of *Eledone moschata* and *Octopus vulgaris* have practically no antidiuretic action (only in massive doses can they reduce or delay the urine excretion). Extracts from kidney, liver, brain, testicle, lung, heart, skeletal muscle and smooth muscle of the stomach and intestine of a calf, and extracts from the smooth gastro-intestinal muscle of a dog are equally ineffective.

It has already been pointed out in the preceding communication that extracts of posterior salivary glands of *Octopus macropus*, which are completely free of enteramine, exhibit an evident stimulating action on diuresis instead of an antidiuretic action.

(2) Provided that care has been taken to eliminate any possible interfering materials (murexine and moschatine, for example), the antidiuretic activity of enteraminic extracts is roughly but clearly proportional, both in intensity and duration, to the content of enteramine, as determined colorimetrically (coupling reaction with diazonium salts in an acid medium, iodine reaction) and biologically (œstrus-uterus of rats or mice, urinary bladder of dogs).

(3) Any procedure which destroys or inactivates enteramine (treatment with formalin, with diazonium salts, with potassium iodate, benzoylation, methylation, ultraviolet irradiation[2]) also destroys the antidiuretic activity of the enteramine-containing extracts. Moreover, the antidiuretic principle of crude extracts closely resembles enteramine in its resistance to alkali and acids in the heat.

Enteraminase (amine oxidase) of fresh phosphate extracts of the intestine, liver or kidney of a guinea-pig completely destroys the antidiuretic activity of the enteraminic extracts, at least so long as the enzyme is not inhibited by methylene blue.

(4) On fractionating dry residues of salivary extracts of *Octopus vulgaris* by various methods, the antidiuretic activity always follows the distribution of the enteramine-like substance. So, for instance, in chromatographic partition experiments on paper[1] and columns, only the eluates from enteraminic spots and zones show diuresis inhibition.

The antidiuretic activity of enteramine-containing extracts has so far been established in rats (the animals on which the bulk of our experiments has been carried out), dogs, guinea-pigs, frogs, toads and humans, both healthy and suffering from diabetes insipidus. Rabbits have given inconclusive results.

Doses of salivary extracts of *Octopus vulgaris*, corresponding to 0·2–0·5 g fresh tissue per kg body weight, have been proved definitely effective in rats and humans.

The onset of action is almost immediate (a few minutes after the s. c. or i. m. injection of enteraminic extracts was given). The urinary block may be total, and lasts for a variable time (up to 5–6 hours or more); it is approximately proportional to the amount of antidiuretic extract injected, naturally within certain limits.

Enteramine inhibits and reduces not only normal and water diuresis, but also that due to xanthines, mercurials, salts and urea.

The hitherto tested enteramine-containing extracts have been numerous and partly obtained from very rich material (about 15,000 specimens of *Octopus vulgaris*, at least as many of *Eledone*, and more than 10,000 specimens of *Murex trunculus*).

Our experiments have been conducted on more than 500 groups of 4–5 rats.

We are indebted to the Italian Research Council for a generous grant-in-aid in connection with this work.

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Pharmacological Institute, University of Bari, July 20, 1950.

Zusammenfassung

Acetonextrakte der hinteren Speicheldrüsen von *Octopus vulgaris* und *Eledone* (*E. moschata* und *E. Aldrovandi*), der Hypobranchialdrüse von *Murex trunculus* und *Murex brandaris*, sowie der Milz und der Magen- und Dünndarmschleimhaut von Säugetieren verhindern merkwürdigerweise die Diurese der Ratte, des Hundes, des Meerschweinchens, des Frosches, der Kröte und des Menschen (gesund und krank an *Diabetes insipidus*).

Die diuresehemmende Wirkung solcher Extrakte ist ausschließlich ihrem Enteramingehalt zuzuschreiben.

Enteramin hemmt nicht nur die normale und die Wasserdiurese, sondern auch die durch Salze, Xanthinderivate und Quecksilberverbindungen erzeugte Diurese.

[1] V. ERSPAMER and G. BORETTI, Exper. *6*, 348 (1950).

[1] V. ERSPAMER e L. PEROSA, Exper. *4*, 486 (1948).

[2] V. ERSPAMER Arch. Sci. Biol. (Ital.) 20, 296 (1940); Acta pharmacol. *4*, 213 (1948).

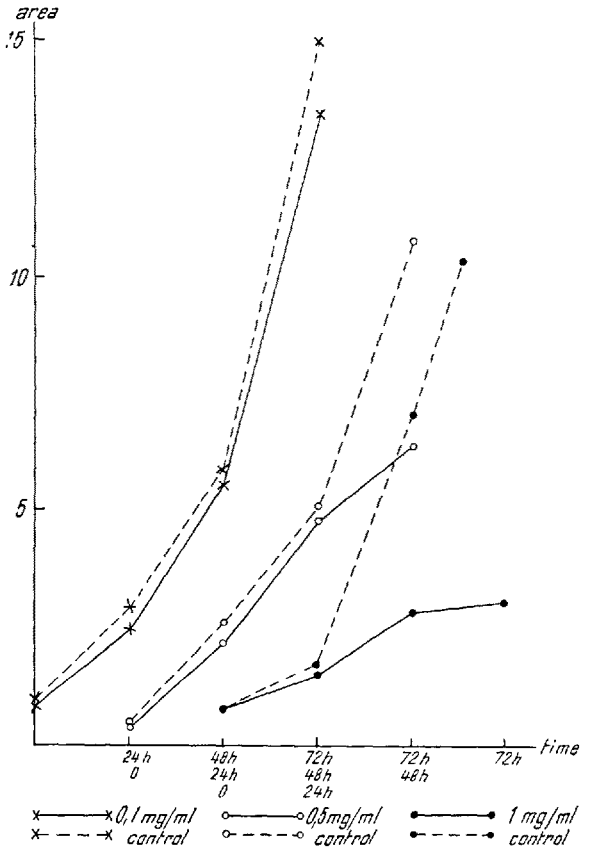
The Effect of Aureomycin on Tissue Cultures

One of the most important of the new antibiotics described in the past few years is aureomycin, which has been isolated from the substrate of *Streptomyces aureofaciens*[1]. The antibiotic has been used mainly in the form of its crystalline golden-yellow hydrochloride, which is soluble in water, but somewhat less soluble in saline. The dilute solutions quickly lose their activity at room temperature and alkaline p_H. Human serum also seemed to have an inhibiting effect on the antibiotic activity[2].

[1] B. M. DUGGAR, Ann. N. Y. Acad. Sci. *51*, 177 (1948).

[2] T. F. PAINE, J. Bact. *56*, 489 (1948).

This antibiotic is active against certain viruses and rickettsiæ and against both Gram-positive and Gram-negative microorganism, while its toxicity to humans and laboratory animals is relatively low[1].



We were interested first of all in determining to what extent aureomycin damages the tissue cells directly, and to what extent it does so by influencing mitosis. The action was tested on explants from the heart and frontal bone of the chick embryo. The technique and arrangement of experiments has been previously described in relation to streptomycin[1], and patulin[2]. Having regard to the inconstancy of the aureomycin solutions, we always prepared the required solutions immediately before carrying out the experiment[3].

In the first series of experiments we added aureomycin directly to the culture medium and followed the growth of the cultures quantitatively. The results are plotted in Table I. In the second series of experiments the cells were in contact with the saline solution of the antibiotic for only 6 hours. A concentration of 0·5 mg/ml caused only some anomalies in the dividing chromosomes. In stronger concentrations of 1, 2, 3 mg/ml a prolongation of the reconstruction and simultaneously a shortening of the prophase was found (Table II). In many cells the division of the nucleus is not followed by the division of the cytoplasm, so that 2–3 nuclear cells appear. Especially at the stronger concentration (3,4 mg/ml) a rather large number of these multinuclear cells is formed, and very often it is difficult to say whether one is dealing with reconstruction or with finished division and that is the reason why the number of reconstructions in the strongest concentrations is relatively low. The cells very often retain their round shape during the whole division and very often also after the division. The nucleus is sometimes pyknotic, sometimes it has little chromatin. In the metaphase pathological anomalies (pyknosis, rhexis) are often found. It is interesting that up to certain concentrations these toxic changes are reversible. Fibroblasts after 12 hours' contact with the solution of aureomycin at a concentration up to 3 mg/ml could be saved by subculturing into a new medium. At the concentration of 4 mg/ml not more than several cells full of fett granules grow out after subculturing. At higher concentrations the damage is irreversible.

In spite of the fact that all these pathological effects appear only at relatively high concentrations of aureomycin, it is necessary to take account of them especially in local therapy.

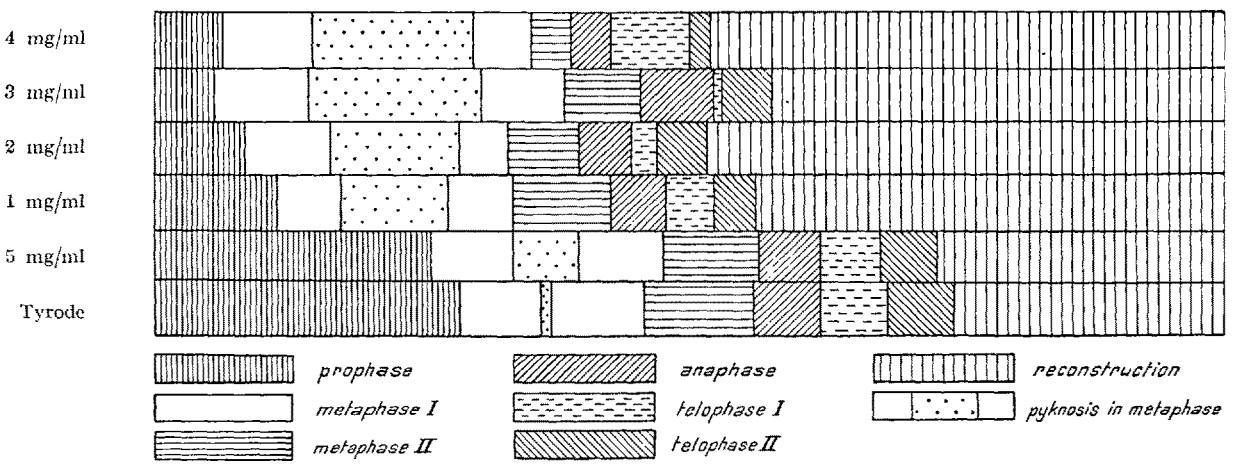
HELENA KEILOVÁ-RODOVA

Department of Animal Physiology, University of Prague, Czechoslovakia, December 14, 1949.

[1] S. MORTON *et al.*, J.A.M.A. *138*, 117 (1948).

[1] H. KEILOVÁ, Exper. *4*, 483 (1948).
[2] H. KEILOVÁ, Exper. *5*, 242 (1949).
[3] An American preparation, Aureomycin hydrochloride, American Cyanamid Company, N. Y., was used in these tests.

Table II



Zusammenfassung

Die Wirkung von Aureomycin auf *in vitro* gezüchtete Bindegewebszellen wurde untersucht. In einer Konzentration von 1 mg/ml und in höheren Konzentrationen konnte eine starke Beeinflussung der Mitose beobachtet werden. Die Rekonstruktionsphase verlängert sich unter gleichzeitiger Verminderung der Prophase. Bei stärkeren Konzentrationen sind die Zellen nicht imstande, die Mitosis zu beendigen, so daß in den Kulturen viele 2-3-nukleäre Zellen entstehen. Bis zur Konzentration von 2 mg/ml sind alle diese Beschädigungen reversibel.

The Utilization of the Branched Chain of Isobutyric Acid Studied with C^{14} [1]

Recent work has shown that isocaproic and isobutyric acids are degraded *in vitro* by kidney or liver enzyme preparations following the classic scheme of β-oxidation. This has been accomplished by manometric measurements of oxygen uptake[2] and by counter-current distribution separation of the reaction products[3]. A mechanism for the formation of propionic acid from isobutyric acid has been proposed[3]. On the other hand, COON and GURIN[4] have reported that leucine is first degraded to isovaleric acid and then β-oxidized to acetic acid and a 3-carbon fragment. It seemed of interest to investigate the behavior of these compounds *in vivo* by the application of radioactive tracer techniques. This paper is a report on experiments carried out with carboxyl- and methyl-labeled isobutyric acid.

Experimental: Sodium isobutyrate-1-C^{14} was prepared by the reaction of isopropyl-magnesium bromide with $C^{14}O_2$ following the directions of CALVIN and coworkers[5] for the preparation of acetic acid-1-C^{14}. The sodium isobutyrate-3-C^{14} was prepared in a similar manner using methyl-labeled isopropyl bromide and inactive CO_2. The specific activity of the sodium isobutyrate-1-C^{14} was 1·72 μc/mg, and that of the sodium isobutyrate-3-C^{14} was 1·50 μc/mg. The yield for the carboxyl-labeled material was 97·5% based on the radioactive barium carbonate employed[6]. The yield for the methyl-labeled material was 48% based on the isopropyl bromide. The preparations were carried out on a 20 mmole scale.

The sodium salt of the acid, ca. 0·075 mg/g body weight, was injected intraperitoneally into a 200 g (Curtis-Dunning strain) rat that had been fasted for twenty-four hours previously. The animal was immediately placed in a metabolism cage, and the expired carbon dioxide, feces and urine collected. This carbon dioxide was collected at specified time intervals in 1 *N* sodium hydroxide and converted to barium carbonate. The specific activity of the barium carbonate was determined according to the method of YANKWICH *et al.*[7]. Geiger-Müller or "Nucleometer" (a windowless proportional counter) counters were used, depending on the specific activities being measured.

After five hours the animal was sacrified. The liver was removed and ground in a glass mortar with sand until very finely divided. The ground mass was fractionated to obtain the total lipid, amino acid and protein, and glycogen.

Discussion: The results of these experiments are summarized in the accompanying figure and table. The curves for the rate of elimination of C^{14} as $C^{14}O_2$ are shown in Fig. 1. The amount eliminated after five hours approaches a value of 80–85% for the carboxyl-labeled compound and 45–50% for the methyl-labeled compound. In the case of the carboxyl-labeled isobutyrate, the final amount and the general shape of curve *A* is similar to the curves observed by JONES and coworkers[1] for the straight chain fatty acids. Based upon the amount of activity of the isobutyrate injected, the ratio of the specific activities of the excreted $C^{11}O_2$ should be 1·82. We see from Table I that the ratio is 1·76.

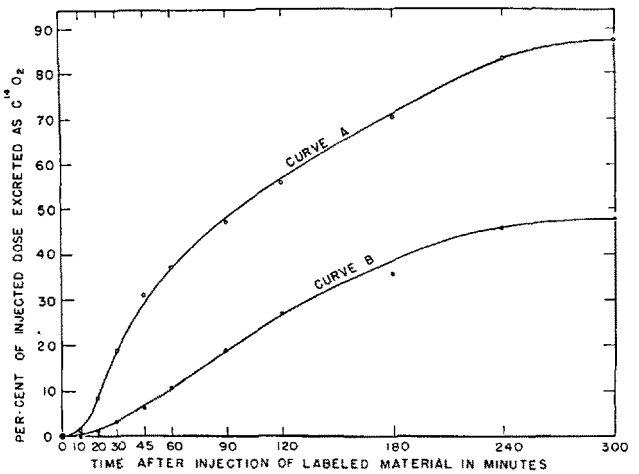


Fig. 1. – Rate of Elimination of C^{14} as $C^{14}O_2$.

There is, however, a definite difference in the initial rates of excretion of labeled carbon dioxide in the breath of the animals injected with sodium isobutyrate-1-C^{14} and sodium isobutyrate-3-C^{14}, that of the animal injected with the isobutyrate-1-C^{14} being the higher.

The difference in these rates together with the amount of radioactivity incorporated into the fraction of the liver leads us to believe that the isobutyrate is degraded to CO_2 and a 3-carbon fragment. This degradation may proceed in either of two ways. The first involves a direct decarboxylation to CO_2 and a 3-carbon fragment, in this case acetone. The second involves β-oxidation to a malonic acid derivative followed by decarboxylation to give CO_2 and a 3-carbon fragment, propionic acid.

If the latter were the major reaction, the propionic acid formed from the carboxyl-labeled isobutyrate would have one-half the activity of that formed from the methyl-labeled material, since it has been shown[2] that propionate is a direct precursor of liver glycogen. The glycogen formed in the liver of the animal given the carboxyl-labeled isobutyrate should have one-half the activity of that from the animal given the methyl-labeled isobutyrate. From the data in Table I we see that the reverse is true.

Assuming direct decarboxylation, all $C^{14}O_2$ excreted in the breath of the animal injected with methyl-labeled isobutyrate would result from further oxidation of the 3-carbon fragment. Since acetone is a symmetrical molecule, half of the CO_2 would be expected to show radioactivity. Actually, 87% of the radioactivity of the carboxyl-labeled isobutyrate and 47% of the methyl-

[1] The work described in this paper was sponsored by the Atomic Energy Commission.

[2] A.L. GRAFFIN and D.E. GREEN, J. Biol. Chem. *176*, 95 (1948).

[3] W.A. ATCHLEY, J. Biol. Chem. *176*, 123 (1948).

[4] M.J. COON and S. GURIN, J. Biol. Chem. *180*, 1159 (1949).

[5] M. CALVIN, C. HEIDELBERGER, J.C. REID, B.M. TOLBERT, and P.E. YANKWICH, *Isotopic Carbon* (John Wiley and Sons, Inc., New York, 1949).

[6] One μc = 2·20 × 10^6 dis/min. The counters used were calibrated using accurately standardized barium carbonate prepared by the Oak Ridge National Laboratories. The efficiency of the counters used was about 5%.

[7] P.E. YANKWICH, Science *107*, 681 (1948). – P.E. YANKWICH and J.W. WEIGL, Science *107*, 651 (1948).

[1] H.B. JONES, personal communication.

[2] J.M. BUCHANAN, A.B. HASTINGS, and F.B. NESBETT, J. Biol. Chem. *150*, 413 (1943).