β-IMINAZOLYLETHYLAMINE A DEPRESSOR CONSTITUENT OF INTESTINAL MUCOSA.

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THE description of a symptom-complex common to the action of commercial "peptone" and of extracts of certain organs, such as intestine, brain, thyroid gland, etc. has formed the subject of numerous papers by Popielski and his co-workers[1]. Lohmann[2] and von Fürth and Schwarz[3] have identified choline as a depressor constituent of such extracts, but, as Popielski and his followers have made clear, the very marked depressant action of these extracts cannot be entirely due to choline, whatever be the final outcome of the discussion as to whether pure choline is depressant. The effects attributed by Popielski to the hypothetical "vaso-dilatin" include fall of blood-pressure, due to vaso-dilatation; loss of coagulability of the blood; violent peristalsis, contraction of the bladder; accelerated secretion of saliva, pancreatic juice and, to a less extent, of bile; depression and narcosis. He regards as fundamental effects the fall of blood-pressure, due to "paralysis of the peripheral vasomotor apparatus," and the abolition of the coagulability of the blood, the other effects being secondary to these. He identifies the "secretin" of Bayliss and Starling with "vaso-dilatin," so that the action of secretin on pancreatic secretion is, according to his view, a secondary and non-specific effect.

Though the repeated association of certain actions is suggestive, it by no means amounts to a proof that they are all produced by a single chemical substance. One of us recently, in conjunction with

[1] Popielski. *Pflüger's Archiv*, CXXVIII. p. 191. 1909. Modrakowski. *Ibid.* CXXXIII. p. 291. 1910.

[2] Ref. *Zentralbl. f. Physiol.* XXII. p. 616. 1908.

[3] *Pflüger's Archiv*, CXXIII. p. 361. 1908.

P. P. Laidlaw[1], described the action of β-iminazolylethylamine and drew attention to the fact that it produced all the effects attributed to "vaso-dilatin" with the exception of that on the coagulability of the blood. Further experiment, of which the details will be published later, has confirmed the absence of effect on coagulability, but has revealed a further point of similarity in that β-iminazolylethylamine increases the flow of lymph from the thoracic duct. Since the purest preparation of "vaso-dilatin" yet obtained by the Lemberg laboratory produced, in a dose of 3·6 mgms., a fall of blood-pressure which, so far as Modrakowski's[2] experiments are comparable with our own, would correspond to a much smaller dose of iminazolylethylamine, it seemed desirable to investigate the possibility that the latter substance might be a constituent of "vaso-dilatin." As in Dale and Laidlaw's paper we shall, for the sake of brevity, refer to the base as β-I.

Having found that all the extracts and preparations in question produce the characteristic action on the non-pregnant uterus of the cat and guinea-pig, we used the response of the latter as a physiological indicator in tracing the active substance. Since intestinal extracts were especially active in producing the vaso-dilator fall of blood-pressure and contraction of the guinea-pig's uterus, we took such an extract for investigation. We were not immediately concerned with the specific effect of secretin on pancreatic secretion, but with the associated depressor action. The highly depressant extract of ox-gut with its comparatively small effect on the flow of pancreatic juice, was, therefore, quite suitable for our purpose.

Small intestines of the ox, fresh from the slaughter-house, were washed through by a stream of water and placed in cold storage at – 2° C. Next day the mucous membrane was scraped off and ground with sand. Enough dilute hydrochloric acid was added to make the concentration of the semi-fluid mass equal to 0·1 % HCl. It was boiled, nearly neutralised, and filtered at the pump. In this way, 750 c.c. of extract were obtained from each kilo of "scrapings."

The extract was concentrated, and 20 % tannin was added to it until the precipitate flocculated. The solution then filtered clear; with excess of tannin this was not so. The filtrate was further treated according to Kutscher's method and the silver precipitate produced by adding excess of baryta in the presence of silver nitrate was collected (precipitates Ag. II and Ag. III). From this the bases were regenerated and extracted with hot absolute alcohol. On evaporation of the alcohol

[1] This *Journal*, XLI. p. 318. 1910. [2] *Loc. cit.*

a syrupy residue remained. This was treated with saturated aqueous solution of picric acid, and yielded 19 milligrams of a picrate which soon crystallised, and then melted at 228° C. On recrystallisation it formed yellow serrated needles and rhombic leaflets, melting at 232° C., which were quite similar to the crystals of β-iminazolylethylamine dipicrate, and like the latter gave an intense colouration with p. diazobenzene sulphonic acid (Pauly's histidine reaction).

The picrate thus corresponded chemically with the dipicrate of β-iminazolylethylamine. For physiological comparison 10 mgms. of the recrystallised picrate were dissolved in dilute HCl and the solution freed from picric acid by repeated shaking with ether. Ether was removed from the watery layer by boiling, and the solution was neutralised and made up to 4 c.c. Assuming that the substance was correctly identified each c.c. would then contain approximately 0·5 mgm. of the base as hydrochloride. This is called solution *A*. For comparison a 0·05 % solution of β-I. hydrochloride, obtained from histidine, was used, and is called solution *B*.

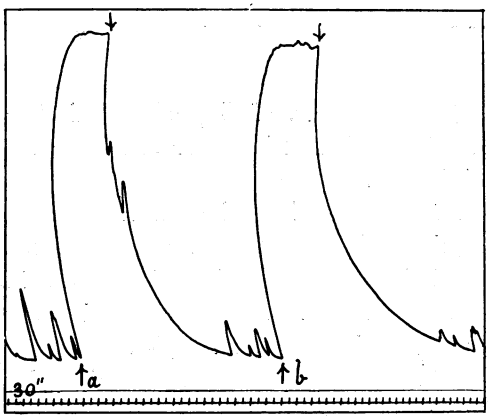

Fig. 1. For description see text. Upstroke = contraction. Fresh Ringer at ↓ ↓.

The following experiments were made:

(1) An isolated horn of the uterus of a virgin guinea-pig was suspended in a bath containing 250 c.c. of oxygenated Ringer's solution at 38° C. Addition to the bath of (*a*) 0·1 c.c. of solution *A* (= 0·05 mgm. of base from intestines); (*b*) 0·1 c.c. of solution *B* (= 0·05 mgm. of β-I.) produced very similar contractions of the uterus (Fig. 1). Solution *A*, presumably containing 0·1 % of base, was as

might be expected, a little more active than *B*, which contained 0·1 % of hydrochloride.

(2) 1 c.c. of solution *A*, injected intravenously into a cat under ether, caused a fall of arterial blood-pressure and increase in volume of a fore-limb (Fig. 2), identical with that produced by the same dose of *B*.

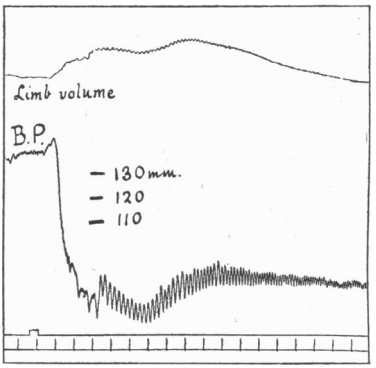

Fig. 2. Effect of 0·5 mgm. of base from intestines on limb-volume and blood-pressure.

(3) 1 c.c. of *A* and 1 c.c. of *B* produced similar rises of blood-pressure in a rabbit which had been for one hour under urethane at the time of the injection.

(4) 1 c.c. of *A* injected into the external saphenous vein of an unanæsthetised guinea-pig caused death from asphyxia in six minutes, with the characteristic permanent distension of the lungs, due to firm contraction of the bronchioles. A precisely similar effect was obtained by Dale and Laidlaw with 0·5 mgm. of β-I.

In physiological action, therefore, as in chemical characters, the base is indistinguishable from β-I., and the identification may be regarded as complete. The yield of picrate which we obtained corresponds only to a small fraction of the activity of the original extract on the blood-pressure or the isolated uterus. The losses, however, due to the unmanageable nature of the bulky precipitates produced, are necessarily very great, and the small yield cannot be regarded as disproving the possibility that β-I. is responsible for practically the whole of the activity in these directions of the original extract. On the other hand it is possible that other substances of somewhat similar action are present.

The hypothetical vaso-dilatin must, therefore, be regarded as consisting of at least two substances:

(1) β-iminazolylethylamine, causing fall of blood-pressure, and the other characteristic effects on plain-muscle and gland-cells, but not affecting coagulation of the blood.

(2) Another substance, or other substances, which render the blood incoagulable and which may or may not play some part in the other effects.

We have no evidence with regard to the origin of the β-I. in the extract of intestinal mucosa. All possible precautions were taken to avoid putrefaction before the material was worked up: moreover a piece of intestine removed immediately after death, or even during life from an anæsthetised animal, washed, scraped and worked up immediately gives an extract with the characteristic physiological action of β-I. Bayliss and Starling[1] showed that the "depressor substance" could be extracted from fresh mucous membrane of dog's intestine by alcohol. It must probably, then, be regarded as a normal product of intestinal mucosa, though whether it is present in living cells, or only formed when these are killed and disintegrated, remains uncertain.

We have made no chemical examination of the similarly acting extracts of other organs, but it seems highly probable that the same base would be found in them. We may mention, in this connection, that we have found the widely varying vaso-dilator action of different samples of "peptone" to run parallel with their activity on the isolated uterus.

It may be desirable to emphasise the fact, already dealt with by Dale and Laidlaw, that this constituent, at least, of "vaso-dilatin," although producing a small secretion of pancreatic juice, has an action in this direction of an altogether lower order than that of an equally depressant dose of secretin. On the other hand, the relatively very weak action of extracts of the large intestine or of "peptone" on pancreatic secretion may probably be adequately accounted for by their content of β-iminazolylethylamine. The effect of the latter on pancreatic secretion is readily abolished by atropine.

SUMMARY.

Popielski's hypothetical "vaso-dilatin" contains β-iminazolylethylamine, which base, however, does not affect the coagulability of the blood.

[1] This *Journal*, XXVIII. p. 335. 1902.