TISSUE EXTRACTS AND INVOLUNTARY MUSCLE.

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THE extensive employment of animal tissues as remedial agents in therapeutics, with a constantly increasing scope of application, makes it important to study not merely the main action of these in effecting their primary purpose, but all collateral changes which might directly or indirectly be attributed to them. The use of hormones or tissue extracts in disease being of comparatively recent introduction, it is but natural that a certain degree of empiricism should be exhibited in the practice of their administration, indications for their selection and use being by no means definite as yet.

It is well known that a hormone may be beneficial to, or have an exciting effect on, one organ or function while it, or some associate of it, may have a depressing or inhibitory effect on another. A particular extract may be capable of influencing several forms of activity; investigation of such differing physiological results has not proceeded very far.

The method of administration of a substance determines rather the intensity than the nature or scope of its action. These now under consideration are for the most part given by the mouth, and for this reason it may be supposed that the stomach and the intestine are amongst the first parts to come under their influence. In these parts it is necessary to take into account two distinct types of activity :

the secreting glands, modifiable in the matter of quantity, quality and rate of flow of digestive juices on the one hand, and the musculature of the walls, modifiable in the matter of tone, rate and force of the contractions on the other hand, require attention. This contribution to the subject is concerned altogether with the latter category of functions. It accrues from an investigation of the changes caused by some extracts in the spontaneous activity of the stomach and cloacal muscle of toads and frogs. The extracts used were those of spleen, lymphatic glands, and duodenum (eukinase).

I am indebted to the British Organo Therapy Company for an abundant supply of these materials, and for their courtesy and readiness to answer all questions relative to the work.

Method.—The simplest means were employed for the examination of the muscle activity and the changes wrought in it by the substances mentioned. The preparations were placed in small glass vessels of 30 to 40 c.c. capacity, with Ringer's solution; one end being fixed to a hook passing through the rubber stopper at the bottom of the vessel, the other end of the preparation is attached to a light lever, which records on a very slowly moving drum. The rate of movement was about one inch per hour; a few of the records have the time marked on them to show slight variations from this. Three or four records can be set going simultaneously. Numerous experiments were made in the investigation—three drums recording together, and two records being sometimes taken on one drum.

It became apparent early in the procedure that the results varied greatly with the strength of the extract employed, so precautions were taken to measure the concen-

tration of the solutions in which the tissue was immersed. The dried extract was taken and pounded in a mortar with a definite quantity of Ringer's solution, which was then added to that surrounding the preparation. The amount of this latter being known, the concentration of the solution was roughly measured, and is mentioned in terms of the weight of dry substance taken and total Ringer's solution. Thus, half a gram of dried extract rubbed up with 5 c.c. of Ringer's fluid, and this added to 5 c.c. R. round the tissue is equal to a 5 per cent. solution. An appreciable insoluble residue always remains, which varies according to the material (it is greatest with lymph gland extract), so that the actual amount dissolved is not represented by the figures.

The first step taken was to ascertain the nature of the spontaneous contractions, the tone, and the "life" in vitro of a large number of specimens in Ringer's solution alone. These vary with many circumstances—the conditions in which the animals have been living, the stage and degree of digestion going on, the method and extent of the handling in dissection, the season of the year, &c. It is impossible to predict in any case the type of record which a preparation will give: the cloaca of one frog, for instance, may be quite different in this respect from that of another from the same tank; even the halves of one cloaca split longitudinally may differ considerably—one half, perhaps, giving vigorous contractions with a rising tone, while the other exhibits slight activity with a falling tone. For this reason it would be quite fallacious to draw conclusions from records which start under the influence of the extract, though this procedure was in some instances adopted. On the other hand, the course of a preparation in uniform conditions never shows those sudden and pronounced changes,

which, following the addition in mid-career of the extracts, may be attributed to the influence of these. The commonest type of record noticed was an initial fall of tone, with slight contractions, lasting a quarter to half an hour or more, followed for a longer or shorter period by a rise in tone, with increasing fundamental contractions. These continue strong for three or four hours, with slight variations in the tone level, which then gradually wanes during a period of from ten to twelve hours. But the life may vary considerably.

It has been shown by Alvarez (1) that rhythmicity in any part of the gastro-intestinal tube varies inversely as the distance from the pharynx. He showed that in the stomach of frogs the lesser curvature exhibits greater activity than other portions. An attempt to differentiate in the present experiments between the effects of extracts on lesser curvature and those on other parts gave no definite result.

Supposing a preparation to conform pretty well to the type mentioned, the best time to test the action of an extract is the period when level tone has been arrived at—that is, about an hour or more after the preparation is set up. This practice was adopted in most instances.

In the earlier experiments, when the solutions were strong, the most striking feature was the drop in tone. In general terms it may be stated that a renewal of tone depended on the period at which the test was made—the longer the tissue was in vitro the less was the recovery. When the extract was tried after several hours, recovery was poor, and the action was, therefore, apparently deleterious; but in the early stages recovery was more pronounced. In the living tissue in situ we may suppose that it would be better still.

There are some well marked differences in the effects of the three substances employed, and there are also differences in the reactions of the stomach and cloaca to them. Generally, the stomach muscle was found to be more resistant to the depressing action than that of the cloaca, which, especially in the case of splenic extract, is very susceptible to the lowering effect.

We shall take each extract separately at first and note its effects with different strengths on the muscle of both regions chosen, and then consider some modifications wrought by combinations of two or more of them.

1. *Eukinase*.—This substance in 10 per cent. solution or over has a distinctly depressing action on the tone of (*a*) the stomach muscle (Fig. 1).^{*} This illustration shows two records, each with a marked fall after eukinase. In the first, it will be seen that the drop is preceded by inhibition of contractions. Only incomplete recovery occurred in these cases. In weaker solution the fall is less, and recovery is good; but the figure, which is typical of numerous other records on the same lines, leaves no room for doubt that eukinase in large doses depresses tone considerably in the stomach. It also causes a fall in tone in (*b*) the cloacal muscle. It is difficult to state definitely that the action is different with this latter muscle from the effect on the stomach; it is somewhat anomalous. We frequently get in this region one, two or more strong contractions following the addition of the extract before a drop in tone sets in (Fig. 2). This figure shows the effect of both a 5 per cent. and 10 per cent. solution on each of two specimens. The individual contractions persist at the lower level, and in the upper tracing (right) they are increased in frequency

^{*} Note.—The curves were demonstrated during the reading of the paper.

during the fall. This type of record is given for the cloaca because it was frequently noted, but in other cases the fall in cloacal tone from eukinase resembled that of the stomach, especially when, individual contractions being naturally small, a strong dose is added at once without preliminary action of a smaller one (Fig. 3). Graduation of the dose also makes a difference in the stomach.

2. *Spleen*.—This extract in the case of (a) stomach muscle has but slight action in weak solution (Fig. 4). This is a record showing some increase of contractions and tone resulting from the action of splenic extract. With stronger solutions of splenic extract the initial fall in tone of stomach muscle is never so marked as in the cloaca. (b) The action of splenic extract on cloacal muscle is always depressing to begin with. Recovery may be prompt (Fig. 5), or long delayed (Fig. 6) and incomplete. As with eukinase, a strong contraction or two frequently precedes the drop in tone of the cloaca following the addition of spleen.

3. *Lymph Gland Extract*.—There has been noticed throughout no marked drop in tone of (a) the stomach resulting from this extract; rather does it cause an immediate excitation, both of separate contractions and tone. In (b) the cloaca it causes usually a preliminary fall in tone with quick recovery, but the amplitude of the contractions may be much less than before.

The stimulating effect of lymph extract suggested a test of another kind—to ascertain if it had any counteracting influence on the depressing action of eukinase and spleen. There seems to be little doubt that it has (Fig. 7). This illustration consists of two records from frogs' stomachs. The first arrow in each case marks the point of the addition of lymph extract, and shows the stimulating action

of this; the second is the point of the eukinase addition (10 per cent.). If this record is compared with those of Fig. 1 a difference in the eukinase effect will be easily observed. The lymph gland extract has apparently counteracted the depressing action of eukinase. In the last illustration (Fig. 8), eukinase is first added in small dose; there is inhibition of the contractions, but it does not prevent the rise of tone due to lymph extract (used at the second arrow). Lymph extract does not counteract the fall in tone caused by spleen in the cloaca. The combination of spleen and eukinase is depressing both on stomach and cloaca.

To sum up the results, we may state that eukinase in large doses has an inhibitory effect on contractions and a depressing action on tone in the stomach and cloaca. Recovery from this is often incomplete *in vitro*. The more efficient recovery in the early stages indicates that a still better recovery might be expected in the living tissue *in situ*; but the results on the whole suggest the advisability of caution in prescribing this remedy in conditions where atony of the stomach is suspected or shown by *x-ray* examination. Small doses of the substance have no marked inhibitory effect which is not quickly recovered from. Of course, we may conceive that temporary inhibition of tone and contractions would be a salutary result in certain phases of stomach activity; and it is further conceivable that the duodenum normally supplies an agent having such an effect, yet it would be set free for use in limited and due proportions. Where atony of the stomach exists, and the administration of eukinase is called for, it certainly seems advisable from these experiments to supplement it freely with lymph gland extract, when large quantities of it are used.

Splenic extract, while it exhibits a marked inhibitory effect on the tone of cloacal muscle with varying powers of recovery, has in the main an excitor effect on stomach muscle, though with large doses there may be in this, too, a slight preliminary inhibition. The temporary inhibition of tone caused by it in the cloaca can scarcely be considered an objection to its free use, as recovery is prompt, and it is frequently succeeded by improved tone.

Lymph gland extract has a stimulating action on both forms of muscle, and from this point of view it would seem to be rather a desirable agent than otherwise.

It is difficult to account for the differences in reaction of cloacal and stomach muscle, which, as stated, are best marked in the case of splenic extract. There is a different nerve supply to the large intestine from that in the small intestine and stomach. The vagus does not supply the large intestine, the activity of which is subject to the action of the *nervi erigentes*, with the sympathetic common to both. We have an analogy in the case of adrenalin, which has an inhibitory effect on the intestinal muscle, while it excites, for instance, that in the walls of arteries. It has been shown (2) that an extract of corpus luteum excites uterine muscle much in the same way that pituitrin does. Corpus luteum and ovarian extract also excite stomach and intestinal muscle, as has been ascertained by Itagaki (3), and in the present series of experiments; extracts of other tissues, voluntary muscle for example, are chiefly depressing in their action.

REFERENCES.

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- (3) Itagaki, Quar. Jour. of Expl. Physiol.