

is quite unknown from the northern parts of Australia or tropical Asia, so that it would appear to have reached Tasmania *via* South America. The entire absence of a lofty mountain range like the Andes, running north and south through the tropics of the eastern hemisphere, has precluded the possibility of a temperate fauna from the north reaching temperate Southern Australia by that route.]

Some Experiments made to Test the Action of Extract of Adrenal Cortex.

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In a recent communication, in which we discussed the significance of secondary sexual characters in the Fowl, we suggested that certain of these characters, *e.g.*, the growth of spurs, "commonly regarded as essentially male, are not attributable to the function of the testicle alone, but possibly indicate the concurrent action of some other gland, perhaps the adrenal, seeing that not a few examples of precocious puberty in children have been found associated with adenomatous or carcinomatous growths of the adrenal gland."†

We selected the cortex for experiment because the new growths occurring in these cases consist of cortical tissue, and because so little is known of the physiological action of the cortex that there was nothing to negative the possibility referred to. The primary object of our experiments was to determine whether the injection of cortical extracts into young animals would hasten the appearance of secondary sexual characters, or exert any influence on the testicle. Since, however, there was no domestic animal smaller than the sheep, in which the male possesses well marked external secondary sexual characters, we were reduced to experiment on birds, selecting the common wild duck on account of the marked difference of plumage in the two sexes and the ease with which the bird can be kept in captivity.

Besides the gradual passage from its nestling to its adult winter and breeding plumage, the male of this species presents a seasonal change.

* The expenses of this research were defrayed by a grant from the Government Grant Committee of the Royal Society.

† "An Example of True Hermaphroditism in the Domestic Fowl," 'Trans. Path. Soc.,' vol. 57, 1906, p. 109. The reason for excluding the testicle in this connection is that the growth of spurs is not inhibited by castration of the young bird.

After the breeding season, that is to say about the end of June, the vividly differentiated plumage of the drake gradually passes into the dusky summer or "eclipse" plumage which renders the drake remarkably like the female bird in the colour of its feathers; this "eclipse" should be complete by about the beginning of August, and after three weeks or a month the reverse change begins to be obvious, the bird gradually reassuming the typical male winter plumage. We did not limit our experiments to the injection of cortical extract into young birds, but we also experimented on birds approaching the "eclipse," and in "eclipse" plumage. The validity of our attempts, however, is marred by the fact that the injections invariably lead to a local necrosis of tissue, which, although it does not obviously affect the health or comfort of the bird, actually produces a delay in the appearance of the secondary sexual characters. The ideal experiment would doubtless be to inject young mammals with the extract from the cortex of adults of the same species; we have already explained why we were forced to employ birds in our experiments. The further departure from the ideal experiment occasioned by the substitution of an extract of mammalian cortex for that of the avian adrenal was necessary for the following reason:—In birds the cortex and medulla of the adrenal gland do not form well differentiated structures as in mammals, but strands of cortical and medullary cells are interwoven throughout the gland in such a way as to make it quite impossible to isolate one from the other. The glands used for preparing the cortical extract were at first procured from goats and used almost directly they were taken from the body, but later on sheep were substituted, and the glands were often kept upon ice for 12 hours before using them; we found no difference in the result. The extracts were prepared with extreme care in order to avoid the possibility of bacterial contamination; the glands with the surrounding fat in which they are embedded were removed from the animal immediately after death and received in a recently boiled, corked or stoppered bottle; they were then taken to the laboratory and used at once, or the bottle was placed on ice until wanted. In either case the fat was removed from the glands with sterilised instruments and the gland immersed in 5 per cent. carbolic acid solution for 10 minutes; it was then washed in boiled salt solution and split into sectors with a sterile knife; the cortex was removed with sterilised curved scissors and the pieces washed in sterile salt solution and pounded with sterilised sand in a sterilised mortar. When the sand and pounded gland had formed a paste, a further quantity of sterile salt solution was added and the paste again treated by grinding and stirring. The brownish fluid obtained by this process was filtered into a sterile beaker through freshly boiled muslin, and the fluid so obtained was injected into the

birds, care being taken to cleanse the skin at the site of injection and to seal the puncture, directly the needle was withdrawn, with collodion.

The usual site of injection was the loose tissue of the fold of the thigh, and although "knots," which we early found betoken necrosis of small masses of fat, regularly appeared within a day or two, the experiments were persevered in since they obviously produced no ill-effect on the general health of the bird or any painful local reaction. Usually the birds received an injection every second or third day. After a month or six weeks we found that the birds so injected not only failed to acquire the male plumage more rapidly, but were, in fact, tardy in assuming it. This applies especially to the young birds in their first plumage. From the similar experiments which we made on adult birds in "eclipse" it can equally be said that the injection produced no acceleration in the change of plumage. We do not consider that the retardation is to be ascribed to any specific effect of the injection, but regard it merely as an example of the general effect on plumage which any slightly unfavourable or unusual circumstance may produce. In the bird the intravenous method of injection is impracticable; and we found that very dilute solutions introduced subcutaneously produce the same necrosing effect as stronger ones. We did not try repeated intraperitoneal injections, for we considered that these could scarcely fail to produce such changes in general health as would tend to retard the normal plumage change. But although these experiments are vitiated by the necrosis produced, it is difficult to believe that none of the material introduced reached the circulation, and they may be tentatively taken to show that the cortex of the adrenal gland does not contain any body which has the specific effect of stimulating the appearance of secondary sexual characters.

We may turn now briefly to the local effects of cortical injection, although these are of small importance as compared with the original object we had in view.

The local effect of injection of a salt extract of the sheep's adrenal is very clearly shown by selecting the pectoral muscle for the site of experiment. A localised necrosis ensues, the necrotic tissue subsequently becoming encapsulated by new-formed granulation and fibrous tissue.

A healthy mallard received 1 c.c. of a salt extract of the cortex of a sheep's adrenals, deep in the substance of the left pectoral muscle, and another cubic centimetre into the loose fold of skin at the base of the right thigh.

The bird was killed seven days afterwards.

In the pectoral there was found a track of dry necrosed muscular tissue lying in a space about an inch in its longer diameter and bounded by a thin newly-formed fibrous capsule.

In the fold of the thigh there was, in the fat, a knot as large as a small haricot, which in section presented an opaque yellow centre and a translucent capsule.

A healthy mallard received into each pectoral muscle 1 c.c. of a salt extract of the cortex of a sheep's adrenals, the cortex used being carefully isolated from the medulla and twice washed in boiled salt solution.

The bird was killed nine days later. In each pectoral there was an encapsulated mass of dry necrotic muscle rather more than 1 cm. in length. In microscopic sections the dead tissue presents the typical appearance of a coagulation necrosis, the muscle fibres being hyaline and structureless. The sequestrum is closely surrounded by a zone of multinucleated giant cells, and beyond this there is newly-forming connective tissue consisting of elongated fibroblasts and intervening fibres, the latter being arranged in correspondence with the form of the necrosed focus.

A salt extract of the *medulla* of the sheep's adrenal likewise produces a local necrosis, on intramuscular injection.

A healthy mallard received into the pectoral muscle 0.5 c.c. of a salt extract of sheep's medulla. It was killed on the tenth day. In the substance of the muscle there was a dry firm sequestrum of muscular tissue surrounded by a fibrous capsule.

That the necrosis of the muscle, so resulting from the injection of medullary extract, need not be explained by a local spasm of the arterioles at the site of experiment appears from the fact that the cortical extract produces the same result. Cortical extract, as is well known from the original work of Oliver and Schäfer, has no immediate physiological effect upon the vascular system.

The necrosis must in both cases be attributed to the direct action of a toxic substance extracted from the cortex and from the medulla.

When similar injections of salt solution extract, whether of the cortex or of the medulla, are made into the tissues of the guinea-pig, oedema or suppuration not infrequently, though not invariably, follow. After injection of medullary extract we have seen extensive cavities containing serous or blood-stained fluid form amongst the muscles.

This result has been described also by Elliott and Tuckett* as following the introduction of pieces of sheep's adrenal, whether medulla or cortex, into the subcutaneous tissue of the same animal, viz., the guinea-pig.

These observers found that no such local results ensued if other animals than guinea-pigs were used to receive the grafts, and this, whether the adrenals grafted were taken from rats, guinea-pigs, or rabbits.

* 'Journal of Physiology,' vol. 34, August, 1906.

They note that this inflammatory reaction at times failed to occur, and in this our results agree.

The conclusion at which the observers named arrive is that the subcutaneous tissues of the guinea-pig are peculiarly sensitive to adrenal grafts, which produce in them œdema and hæmorrhagic solution.

It is difficult, however, to eliminate here the possibility that the results are due to auto-bacterial infection.

The organs of normal guinea-pigs abound in various forms of bacteria, the growth of which is inhibited under natural conditions. When necrosis is set up by the adrenal graft or by the injection of an extract, the dead tissues furnish a nidus in which the latent pathogenic micro-organisms may grow.

Further Results of the Experimental Treatment of Trypanosomiasis: being a Progress Report to a Committee of the Royal Society.

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The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'*

The experiments have been carried out with the same strains of Nagana and Surra as were used before.

A.—Condition of the Animals living at the Date of the Completion of the Tables in the last Paper.

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury.

(Average duration of untreated disease, 5·5 days.)

No.	4	died on the	307th	day after inoculation.
„	7	„	365th	„
„	10	„	249th	„
„	15	„	188th	„
„	21	„	63rd	„

Of these, No. 15, which was apparently cured, was used on the 147th day after inoculation for re-inoculation, with the view of ascertaining if any immunity had been conferred. This was found not to be the case.†

* B, vol. 79, 1907, pp. 505—516, and B, vol. 80, 1908, pp. 1—12.

† *Vide* 'Roy. Soc. Proc.,' B, vol. 80, p. 10.