extract but in a form too readily inactivated in the body, the extract was prepared as above and treated as follows: The breast of a normal chicken was inoculated repeatedly with kieselguhr, which is known to stimulate a mononuclear and giant cell response. After two days the yellow granular exudate thus produced, together with a small amount of muscle, was scraped out and a 10 per cent. suspension prepared by grinding with sand in normal saline. The suspension was centrifuged at high speed and the supernatant fluid separated. The benzene extract of the tumor which had been evaporated to a sticky mass was then suspended in this saline extract of normal chicken wandering cells and muscle, incubated one hour under nitrogen at 37° C. and injected immediately. Large tumors with many metastases were produced, and these were capable of transmission for an indefinite period. Again repeated inoculations were required.

Subsequent studies were aimed at the isolation of a lipid fraction, by methods less injurious to the active agent. The acetone technique was discarded and the ground tumor tissue was frozen with carbon dioxide snow and desiccated in the Flosdorf-Mudd Lyophile apparatus. The dried material thus prepared shows no loss of activity. Upon removal from the high vacuum apparatus, the dried tumor was immediately extracted with benzene, or, at a later date, with carbon tetrachloride, in a Soxhlet as before. This extract gave negative results if injected alone, but when incubated with a saline extract of chicken muscle prepared as above, produced large tumors more rapidly than those hitherto obtained. This experiment has been repeated on several occasions during the past year, since the procedure was utilized as a control for further studies, and the extract thus prepared has been found capable of producing tumors within two to three weeks following a single injection. Other chickens were given two or three injections. Almost 100 per cent. of the chickens developed tumors, and the majority appeared to be highly malignant.

The residue of dried tumor after extraction with benzene still retained a degree of activity, which was to be expected, since extraction with benzene or carbon tetrachloride is less complete if the tissues have been previously frozen and dried than if acetone is used as the dehydrating agent. The disadvantage of the material waste was more than compensated for by the superior activity of our final fraction.

A series of animals was subjected to a slight modification of procedure. Kieselguhr was inoculated into the breast muscle several times until considerable injury had occurred, and the lipid extract of the tumor was then injected alone. Tumors did not develop, showing that a period of contact with normal tissue extract *in vitro* is necessary for the production of tumors.

To test the possibility that the active agent was a contaminant introduced by contact with instruments or glassware used in the routine transplantation of the tumor or that a ubiquitous agent existed, capable of acting upon tissues prepared by the inoculation of these lipid fractions, the same procedure was followed, using the lipid fraction of normal chicken muscle. This extract obtained after freezing, drying and extraction with carbon tetrachloride failed to produce tumors alone or following incubation with a saline extract of muscle. The same precautions as to temperature and the use of nitrogen were observed throughout the extraction, and the material was injected immediately. Repeated inoculations were given and the animals kept for an equal period of time.

The lipid extract of the Rous chicken sarcoma No. 1, then, obtained by use of a specific solvent at low temperature under relative anaerobiasis, was capable of transmitting the tumor to chickens when allowed contact with chicken tissue extract for a brief period and inoculated promptly. Tumors did not develop if the lipid extract was inoculated alone or into previously injured breast muscle.

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SOME EFFECTS OF ANDROGENIC SUBSTANCES IN THE RAT[1]

A. THE EFFECT OF MALE HORMONE EXTRACTS ON THE TESTES OF HYPOPHYSECTOMIZED RATS

WALSH, Cuyler and McCullagh[2] reported that the daily administration of extracts of male hormone prepared from urine would prevent the usual atrophy of the testes that occurs following hypophysectomy in the rat. In their experiments injections of male hormone (9.0 B.U. per day) were instituted the day after hypophysectomy and continued for three weeks. To our knowledge this amazing finding has never been confirmed or denied.

We have recently made a series of experiments in which a comparison has been made between the effects of castration and hypophysectomy on the reproductive system of male rats (4 to 5 months old). As an extension of that study it has seemed desirable to compare the effects of male hormone extracts on the reproductive systems of such animals. The maintenance of the various sex accessory structures has

[1] These investigations were aided by grants from the Anna Fuller Foundation, the Fluid Research Fund of Yale University, and the Committee for Research in Problems of Sex of the National Research Council and the Rockefeller Foundation.

[2] E. L. Walsh, W. K. Cuyler and D. R. McCullagh, *Am. Jour. Physiol.*, 107: 508, 1934.

been quite similar in the two conditions, a finding that was not surprising.

In our earlier experiments we employed a dosage of 10 B.U. per day begun the second day after hypophysectomy and continued for 20 to 21 days. Although the testes of the animals in this series were much smaller than normal, they were definitely larger than those of untreated controls. Consequently, it seemed desirable to continue these experiments with higher dosages. Table 1 shows the details of the experiments together with the weights of the testes, as averages for each group.

TABLE 1

THE EFFECT OF MALE HORMONE ON THE TESTES OF HYPOPHYSECTOMIZED MALE RATS

No. of rats	Average weight	Bird units of male hormone per day	Period of hypophysectomy	Period of treatment	Weight of testes	Sperm motility
	grams		*days*	*days*	*grams*	
21	267	..	Normal	.....	2.432	Excellent
8	208	..	22–23	.....	.517	No sperm
5	197	10	22–23	20–21	.801	Fair
5	189	14	22–23	20–21	1.031	Good
6	199	20	22–23	20–21	1.609	Excellent
2	212	20	40	38	1.586	"
3	195	..	40	.....	.423	No sperm

It will be noted that even in the 20 B.U. group the testes were smaller than normal. However, they were over 3 times as large as those of the untreated controls and were turgid rather than flabby. In histological section the testes of the treated animals showed seminiferous tubules that were entirely normal except for a decrease in diameter of about 20 per cent. Spermatogenesis was progressing in an apparently normal fashion. Sperm motility tests have shown that, while sperm were no longer present in the epididymidi of animals hypophysectomized for 22 days, the injected animals had normal sperm motility. As far as we have been able to detect the degenerative changes that occur in the interstitial cells following hypophysectomy have not been prevented.

Two males were hypophysectomized and injections of 20 B.U. daily continued for 38 days. The testes of these animals were almost 4 times as large as those of the untreated controls (Table 1). One of these males sired two litters of rats (on the 31st and 39th days after hypophysectomy), and the other, one litter (on the 36th day). This is of particular interest, since it not only demonstrates that the sperm possessed the capacity for fertilization, but also that male hormone extracts can restore libido in hypophysectomized animals.

At the present time a group of males have been under treatment with male hormone for six weeks following hypophysectomy. It is intended that they will be so treated for at least 60 days.

Five hypophysectomized males were allowed to remain untreated for 21 to 24 days. At that time one testis, one seminal vesicle and a piece of the prostate were removed and injections of male hormone (20 B.U. daily) were started. Although the sex-accessories were completely repaired, there has been no detectable effect of the male hormone on the testes of these animals. The testes of other animals on a similar schedule, but treated with pituitary extracts, were returned to a normal condition.

Hypophysectomy was complete in all the animals reported here as shown by sections of the sella turcicae and by conditions of regression in the thyroid and adrenal glands. The extracts used in the experiments reported here were highly purified concentrates of male urine. We feel reasonably certain that the possibility of the extracts being contaminated by gonadotropic hormone is exceedingly remote in view of the extraction procedures involved in their preparation. However, experiments to test the action of various synthetic androgenic substances on the testes of hypophysectomized rats are now in progress.

B. The Effect of Some Synthetic Androgenic Substances on the Hypophysis, Mammary Gland and Uterus of the Spayed Rat

Nelson and Gallagher[3] have shown that male hormone preparations obtained from either urine or testes will prevent the occurrence of castration changes in the hypophysis of the rat. Although preparations from which oestrogenic activity had been removed by special procedures were effective it seemed important to investigate the action of synthetic androgenic substances on the hypophyses of castrated rats.

Three substances, *viz.*, androsterone, androstanediol and androstene-dione, have been injected in spayed virgin rats. Injections have been made at two levels, 0.5 mg and 1.0 mg daily over a 30-day period, beginning the day after ovariectomy. Vaginal smears were taken daily and at the close of the experiment hypophysis, uterus, vagina, mammary gland, adrenal and thyroid have been removed for study.

Androstane-diol and androstene-dione at the 1.0 mg level not only suppressed the castration changes in the hypophysis, but induced the same type of degranulation that has been reported in animals injected with oestrin by Nelson.[4] The 0.5 mg level was not completely effective. Androsterone at the 1.0 mg level almost, but not quite, prevented castration changes.

[3] W. O. Nelson and T. F. Gallagher, *Anat. Rec.*, 64: 129, 1935.

[4] W. O. Nelson, *Proc. Soc. Exp. Biol. and Med.*, 32: 452, 1934.

The mammary glands of the animals on the 1.0 mg level of androstane-diol showed a remarkable proliferation. The development of the ducts was complete and many lobules were present. All acini showed definite secretory activity, but not lactation. Androstene-dione at the 1.0 mg level showed a similar, but less marked effect. Androsterone exerted no detectable influence on the mammary glands.

The uteri of the animals injected with androstane-diol (1.0 mg) showed a marked increase in connective tissue and smooth muscle and were not only much larger than the uteri of castrate controls, but were larger than at the beginning of the experiment. The uteri of the animals that received androstene-dione showed a slight stimulation, while those of the androsterone series were typically castrate.

The vaginal smears of all animals were consistently dioestrus throughout the experiment. In section the vaginas of the animals injected with androstane-diol and androstene-dione showed a slight mucification, while those of the androsterone-treated animals were typically castrate.

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FIXATION OF POTASSIUM IN SOILS[1]

FOR more than half a century the problem of K fixation in soils was investigated and discussed. It was noted that when soluble potassium was added to the soil a large portion of the K became unavailable. With the clarification of the phenomena of base exchange the immobilization of soluble K in soils was considered from the standpoint not only of its insolubility in H_2O, but also in the sense of its being non-replaceable. A number of postulates have been suggested on the mode of fixation. It was natural to suspect the silicates, the primary source of K in soils, as the seat of reactions involving fixation of the K added or released in soils. The one postulate which gained popularity in recent years was that the soluble K in soils reverts to a difficultly soluble complex resembling muscovite. No definite evidence to prove this contention has been advanced. It is perhaps somewhat far fetched to think of the formation of silicate minerals of K under conditions of temperature and pressure prevailing in the soil.

In a series of experiments, conducted by the authors, with artificially prepared silicates of various ratios of SiO_2/basoids subjected to alternate wetting and drying, no fixation of K could be demonstrated. The attention was then directed to other acidoids and it was found that the phosphates of a number of cation linkages are capable of fixing K in unavailable or non-replaceable form.

Aluminum and iron phosphates were prepared and treated with solutions of KCl corresponding to applications of 7.6 per cent. of the total dry weight of the respective phosphate complexes. These systems, prepared in triplicates, were then alternately wetted and dried five times at 23°, 35° and 70° C. The complexes dried at 70° C. fixed the largest quantities. The iron phosphate fixed 72.15 milliequivalents of K per 100 grams, which represents 57.85 per cent. of the total KCl applied, and aluminum phosphate fixed 71.43 milliequivalents, which represents 57.14 per cent. of the KCl applied.

Other cation linkages have been tested under various conditions and they also were found to fix the K.

There is an indication that the NH_4 ion and perhaps other cations may be fixed in the same manner.

Pedological data on hand seem to fit in with the findings of the laboratory experiments on the fixation of K through the medium of phosphated complexes. There is a definite relation between the phosphated complexes of various cation linkages and the extent of K fixation.

A more detailed description of the data on hand, the probable chemical reactions involved in the mode of fixation of K by the phosphate complexes, and the implications involved with respect to systems of fertilization will be dealt with in a more extensive manner in a paper to be submitted to *Soil Science*.

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NEW JERSEY AGRICULTURAL EXPERIMENT STATION

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW TECHNIQUE FOR PRODUCING LESIONS OF THE ENCEPHALON CORTEX

TECHNIQUES for producing controlled lesions of the encephalon that are uniform in depth and limited to the cortical layers are comparatively crude and unsatisfactory. This is particularly the case when large lesions are desired and when chronic preparations are necessary. With small animals such as the rat, added difficulties arise from the spatial limitations of the small field of operation.

By adapting the copper point of an electric soldering iron, Dusser de Barenne[1,2] has devised probably

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

[1] J. G. Dusser de Barenne, *Zeit. f. d. Ges. Neur. u. Psychiat.*, 147: 280, 1933.