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THE TESTICULAR HORMONE.*

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Preparation.

The preparation of testicular extracts has for the last 3 years been subjected to intensive investigation by this laboratory. McGee (1) and McGee, Juhn, and Domm (2), have shown that extracts of the lipid fraction of bull testicles exert a striking effect on the secondary sex characters of the Brown Leghorn capon. Gallagher (3) has shown that by the present methods of extraction and assay this activity is found in no other tissue save testis and epididymis. Moore and McGee (4) have shown these extracts to possess the same activity as the internal secretion of the testis by their effect on the spermatozoa in isolated epididymides according to the criterion proposed by Moore (5). These results in the mammal have been extended by Moore and Gallagher (6) with more highly purified preparations and some attempt has been made to investigate this reaction as a means of quantitative assay. A further new series of indicators has been proposed by Moore and Gallagher (6, 7), Moore, Price, and Gallagher (8), and Moore, Hughes, and Gallagher (9), based upon cytological changes in the accessory reproductive organs and the secretion of the seminal vesicles and prostate gland.

These varied biological manifestations of the testis hormone have been developed by the Chicago group in hope of attaining some rapid quantitative method of assay for testicular extracts. An extensive investigation of the comb growth reaction in the

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Brown Leghorn capon has been undertaken by Gallagher and Koch, which will be the subject for a future communication. It will suffice here to note that while this reaction admits of certain quantitative interpretation it is by no means as accurate as desired. It fulfils however the requirement of rapidity in that but five daily injections are required.

In the studies reported on in this paper the routine assay has been the injection of the extract once daily for 5 days. We wish to thank Dr. L. V. Domm of the Whitman Laboratory of Experimental Zoology for preparing the capons used in these investigations. Comb measurements are taken on the 1st, 3rd, and 6th days and results interpreted on the basis of the preliminary standardization of the extract studied. It must be emphasized that proper interpretations can be made only if a minimal dose be determined and all subsequent studies be based on this minimal dose.

The method of preparation proposed by McGee has been investigated and adopted by us as the first step in the routine extraction.

The tissue is ground and extracted with 4 volumes by weight of 95 per cent alcohol for from 3 to 5 days. The alcoholic extract is pressed out, concentrated to a sludge under diminished pressure, and extracted with benzene.

In view of the paucity in yield of activity per unit weight of tissue we investigated at some length the completeness of our alcoholic extraction. Since reextraction of the tissue residue with 95 per cent alcohol yields very little or no activity, we have found no justification for reextracting the tissue as a routine precaution. Womack in this laboratory is extending these results.

Another explanation of the low yield suggested itself; namely, that the active principle might be bound in the tissue in such a way that the activity might not be recovered completely by a simple extraction process. Hydrolysis with strong alkali, acids and enzymes was investigated but in no case could the yield be increased. There was often some loss, especially with alkali treatment, but it is significant that fresh bull testis may be boiled with 40 per cent NaOH for 2 hours without too great loss of activity. This finding however does not disprove that the active principle is in some way bound, for, since there was some destruc-

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tion, there may have occurred simultaneous release and then destruction of the activity.

The benzene-soluble material as obtained by the procedure adopted by McGee (1) is unfit for continued injection due to the severe local reaction produced. The most satisfactory treatment consists in complete removal of benzene by distillation under diminished pressure and treatment of the residue with acetone. The complete removal of benzene is of great importance. We have repeatedly noted that if the removal is not complete the yield of solids obtained in the acetone varies considerably and the complete removal of activity from the lipid precipitate is not obtained. The acetone-soluble material contains the greater part of the activity. Some slight activity may be recovered by a second extraction of the acetone precipitate, but when this is done, the activity per unit weight is distinctly decreased. The material obtained in this step is suitable for repeated assay in the bird but is toxic in small doses to the mammal. The yield is usually of the order of one-tenth the total weight of the benzene-soluble substance and the minimal daily dose in the capon of the product varies from 20 to 30 mg. The details followed in an actual experiment are given below.

TABLE I.

Assay of Preparation 84.

Preparation.	Weight of yield.	Dosage.	Assay.
	gm.		
Alcohol-soluble.		Very large, toxic.	None made.
Benzene-soluble.	211	" " "	" "
Acetone-soluble.	20	0.010 gm.	Negative, 3 birds.
		0.015 "	" 3 "
		0.020 "	" 3 "
		0.030 "	Positive, 3 "
Acetone-insoluble.	191	0.15 "	Negative.

Preparation 84.—29 kilos of tissue were extracted with alcohol, concentrated, and extracted with benzene as outlined. The benzene-soluble material weighed 211 gm. This was extracted with 1 liter of acetone at −10° for 24 hours. The acetone-

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soluble material weighed 20 gm. Table I gives the results of the assay. The minimal daily dose hence is the equivalent of 43 gm. of frozen bull testicle.

This material is rich in cholesterol and neutral fat. Several alternative procedures have been investigated for further purification. Precipitation with dilute alcohol suggested itself. By using 50 per cent alcohol at −10° roughly 96 per cent of the solids may be precipitated. The filtrate is highly active, the minimal dose varying from 0.2 to 2 mg., dependent upon conditions which we shall discuss. The recovery in activity however is not entirely satisfactory, varying from 40 to 60 per cent. Even after five extractions of the precipitate much activity still remains. The principal difficulty is met in the physical state of the precipitate. Emulsions form which presumably adsorb the active substance and a layer of liquid fat usually separates which tends to hold back the hormone.

70 per cent alcohol at room temperature offers greater advantage. The yield in solids is about 40 per cent, but the recovery of activity is fairly complete. The material may be further purified by using smaller volumes of alcohol and repeating the procedure with the soluble material. Here, however, the yield in activity begins to fall, complete recovery in the desired fraction being impossible. Since these procedures have been discarded, for the sake of brevity, no typical experiments will be given here.

Various experiments involving partition between immiscible solvents were undertaken, the most satisfactory one being a separation of the acetone-soluble material between 3 volumes of 70 per cent alcohol and 4 volumes of hexane. The yield in solids is approximately 4 per cent and the recovery of activity from 70 to 80 per cent. We have accepted this loss in view of the relatively great purification attained.

The material now is dissolved in ether as well as possible and washed with 10 per cent NaOH. The yield in solids in the ether phase is from 15 to 20 per cent and as far as we can determine complete activity is recovered. Details of a typical experiment are given.

Preparation 89.—36 gm. of acetone-soluble material obtained by the routine procedure were dissolved in 175 cc. of hexane and then shaken with 125 cc. of 70 per cent alcohol. The 70 per cent

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alcohol layer was washed five times with hexane. The total hexane-soluble material was then reextracted twice with 75 cc. of 70 per cent alcohol and in each case the alcohol layer was washed five times with fresh hexane. The 70 per cent alcohol-soluble material after removal of the alcohol was transferred to ether and shaken with 10 cc. of 10 per cent NaOH. The NaOH solution was washed five times with fresh portions of ether and the ether solution shaken repeatedly with water. The ether-soluble material is then suitable for assay. The result of the assay is shown in Table II.

The material thus obtained may by suitable treatment be rendered water-soluble, but our data are as yet incomplete and we prefer to postpone communication on this phase of the problem.

TABLE II.

Assay of Preparation 89.

Preparation.	Total weight.	Daily dose.	Assay.	Recovery of activity.
	gm.			*per cent*
Acetone-soluble.	36	0.03 gm.	Active.	
		0.02 "	Inactive.	
Hexane-soluble.	34.8	0.10 "	"	
		0.15 "	Trace of activity.	15 (?)
70 per cent alcohol-soluble.	1.28		None made.	
Ether-soluble.	0.285	0.28 mg.	Active.	85 (?)
		0.50 "	Strongly active.	

Further purification may be obtained by precipitating the ether-soluble material with small quantities of a low boiling hydrocarbon such as pentane. There is however always a decided split in the activity, and a consequent low yield. This is rather to be expected since our data indicate a rather low solubility of the hormone in such solvents.

DISCUSSION.

The method as outlined has certain features both in its favor and against it. The long treatment and difficulty of handling such extractives quantitatively speak against it. On the other hand, a potent preparation in fairly good yield may be obtained.

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The material upon repeated injection in the bird is certainly non-toxic and in the mammal even huge doses elicit no unfavorable local reaction.

The solid content of the daily minimal dose for the capon may be reduced as low as 0.01 to 0.03 mg. if complete recovery of activity is not desired. In such a case the volume of 70 per cent alcohol used may be reduced and quite a difference in the yield of solids effected. In many chemical studies we have preferred to emphasize purity of product rather than completeness of yield. These will be a subject for future communication.

Whether one or more active principles are concerned is at present mere speculation. It is however true that by one and the same method of preparation an extract is obtained which will exert all the biological effects thus far postulated for the testis hormone. Physiological and morphological criteria have been proposed and in every case complete substitution may be obtained with the testis extract here described.

It is our feeling that until more is known of the chemical nature of the hormone no name should be given the extract. As yet any name would be valueless and not at all descriptive. Too often a name gives a false sense of security as regards the purity of the product, a fact we wish to emphasize, for it is our firm opinion that the extract is as yet grossly impure even though the minimal daily bird dose be as low as 0.01 mg.

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