The seminal vesicles of the castrated mouse, test for the testicular hormones (1)

By THALES MARTINS, M. D., and A. ROCHA e SILVA

For some eight months past, we have been going into the question of tests for testicular hormones. Our object was chiefly to study the changes in seminal vesicles of white mice, as their atrophy after castration is a well known fact. (STEINACH (1)). We had in view determining which were the earliest changes found after gonadectomy; this being ascertained, the second part of our task was to investigate whether these changes might be reversible under the influence of injections of an active substance.

One may say that, as a rule, the atrophy of seminal vesicles in castrated mice easily allows, after 9 to 11 days, to detect a castrated from a normal mouse.

Normally the mucosa is covered with multiple villosities anastomosed in some spots. The cells are of the cylindrical type, the nucleus being situated at the basis of the cell.

The cytoplasm is rich in granulations. The lumen of the vesicle is filled with a viscous fluid, of milky appearance. We never found spermatozoids in the vesicles of normal white mice.

Five days after the castration, the morphologic changes are quite visible: reduction of the cytoplasm, nucleus in the centre of the cell.

The cytologic structure is modified; GOLGI apparatus, secretion granules. The cells assume a cuboidal shape, and the granulous aspect of the cytoplasm disappears, becoming homogenous. After a longer time, these changes become increasedly marked; the villosities lose their characteristic appearance and atrophy themselves.

The contents of the vesicles pass gradually to form a transparent fluid. The changes in the prostate, in the deferent canal and the penis do not present equal interest; therefore we leave them aside.

After having castrated more than 200 mice, of which we examined more than 115, we have come to the conclusion that the macroscopic sizes of the vesicles and cytological changes constitute a satisfactory test with regard to testicular hormones.

We are endeavouring to make it easier, by simplifying the histological technique, in order to get by means of dissociation or smearing, a ready verification of cytological changes, avoiding the long manipulation of inclusion, cuts, etc., of the material.

The best fixating mixtures are those that contain osmic acid.

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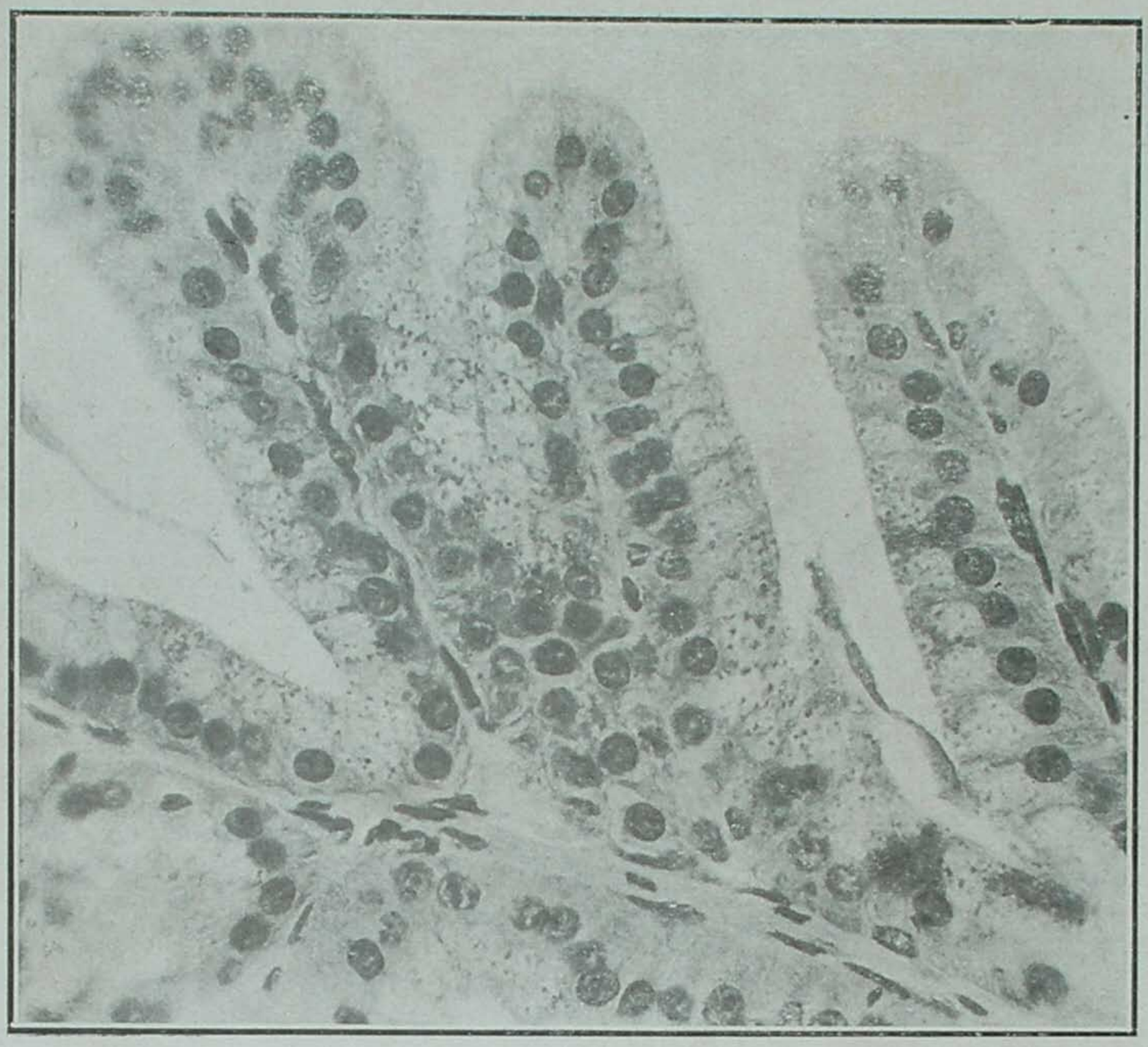

Fig. 1—Seminal vesicle of normal adult white mous. 1:600

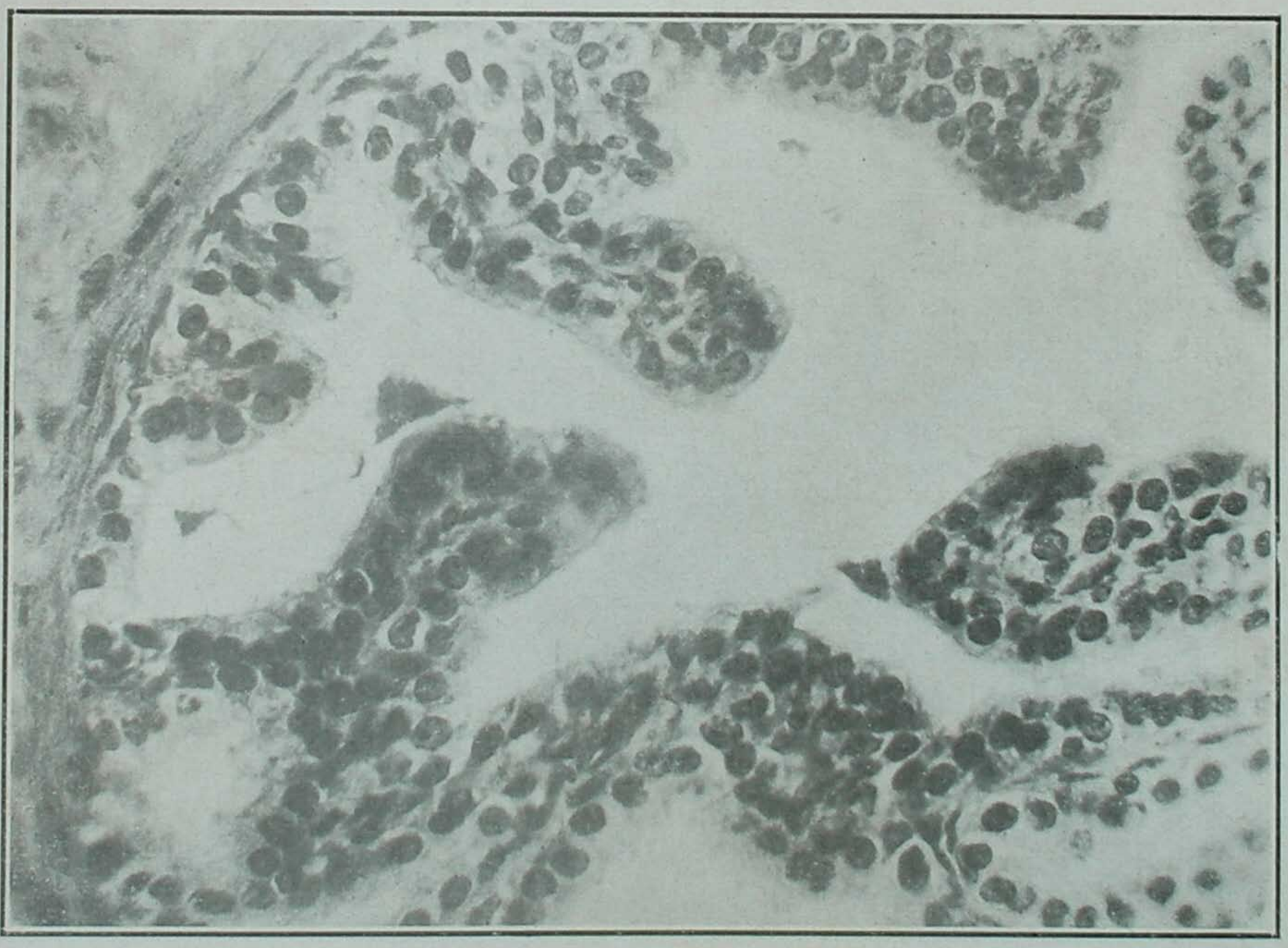

Fig. 2—Seminal vesicle of adult mous, 11 days after castration. 1:600

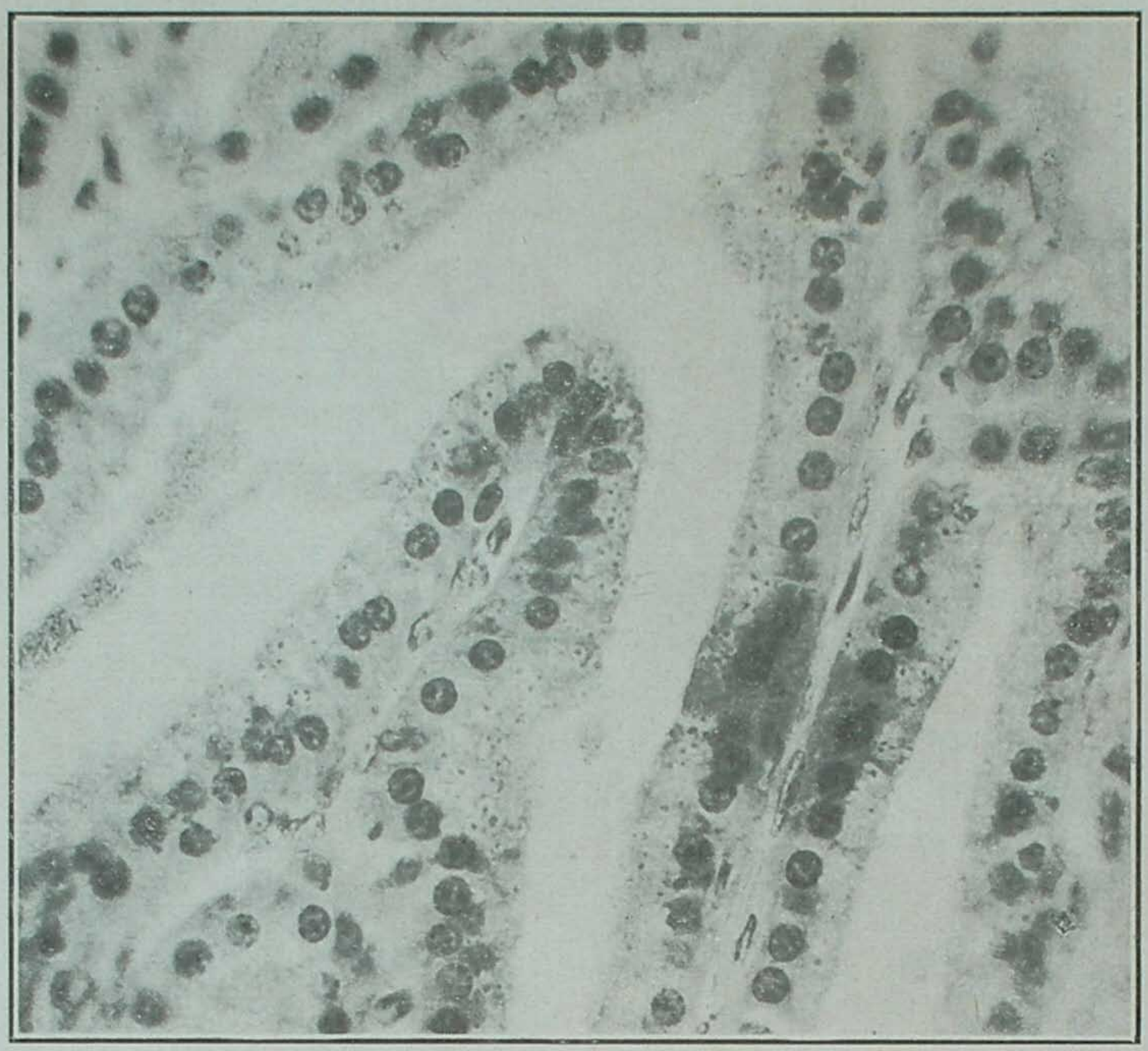

Fig. 3—Seminal vesicle of adult mous, 11 days after castration and injection of lipin extracts of goat testis. 1:600

1 2 3 4

Fig. 4—Vesicles of 17 grs. mice, same litter.

1—normal.
2 and 3 - 11 days after total castration.
4 – 11 days after castration, with conservation of the epididymis.

MEASURES

We note preferably the maximum length and width ; the product of both (L × H) gives an empirical number, which we shall admit to represent a size index, the same as Mc GEE (2) did for the capon comb. The weight is not so reliable an element. Numerous are the causes of errors. MUTO (3), who used to note the weight of associated glands as a guide for his researches on testicular hormones, reports most varying results.

Atrophy is more regular in still young specimens weighing 17 grams more or less. In such animals, we found, as a rule the length about 14 mm., and width 3,7 to 4,5 mm., and the index (L × H) 53 to 63).

In castrated animals, the vesicles, measured by the 10th to the 13th day after the operation, show an index in the vicinity of 20, not seldom under 10.

RESULTS OBTAINED BY INJECTION OF EXTRACTS.

These facts being established, we undertook then, to verify the influence of the injections of extracts. The injections were effected from the day immediate to castration, during a period of 5 to 10 days. One understands easily that the threshold to prevent atrophy taking place, should be lower than that necessary to regenerate an organ already in complete involution.

We used at the beginning suspensions or macerations of freshly extirpated testicles of mouse and rat. The results were not bright at all. Even when we injected subcutaneously to the animal, its own testis extirpated on the spot, the vesicles after 10 days were hardly dfstinguishable from the uninjected controls. We further injected testicular lipoids of bull and goat. We succeeded thus in obtaining unquestionable results: not only the cytoplasmic granulations were still richly prevailing, as also the size of the organ was, as an average, by 100 o/o higher than in controls.

With active extracts, we obtained index numbers up to 60, whilst the highest index for controls was 23, and together with this, the complete maintaining of the normal cytological appearance.

We may conclude that the intended test is quite satisfactory, and may be adopted, as long as no better one is proposed. Taking it as a basis, we shall admit as unit for testicular hormones (at least the hormone that has an influence on accessory glands), the smallest quantity which, when injected daily for a period of 10 days, keeps the seminal vesicles of the castrated white mouse at a level close to the normal, so as to keep the cellular characters of the gland unchanged, and to obtain for an index as described above, a number at least higher by twice than that found in the castrated controls of same weight and time of castration. This will prevent remaining within the limits of possible errors.

Of course, by further study, other details may be established, which will allow of an easily reaching a simple standard.

Ours is at least easier and quicker than the test of capon comb, chiefly used by Mc GEE, and of spermatozoid motility of MOORE (4) which seemed to be, up to now, the most reliable.

We presently used the test of seminal vesicles, not only in view of

the question of testicular extracts, but also for the study of other connected problems.

With implantations of the fore-lobe of the hypophysis of rabbit, effected twice a week, during a period of 2 to 3 months, in very young or normal adult mice, it is not possible to obtain in the animals, an index higher than the normal.

One may observe that they reach sizes and a structure rather approaching the maximum figures found in normal animals.

Also by a longlasting series of injections of hypophyseal extracts from pregnant women urine, the results are the same.

This is a coutribution to the study of the "law of all or nothing" as described by PEZARD (5), in fowl, and by LIPSCHÜTZ (6), in rodents.

Noteworthy is the fact that, when one or both epidydimides are left in the gonadectomized animals, the atrophy of the vesicles is delayed in many cases.

This does not happen in all cases, but is frequently observed. We understand it as representing a storage of testicular hormone, which is reabsorbed after the castration. It is obvious that care was taken, not to leave testicular fragments sticking to the epidydimis.

Our observations were ready for printing, when we received yesterday the "American Journal of Physiology" LXXXIX, N. 2, containing an article from MOORE and GALLAGHER "On the prevention of castration effects in Mammals by Testis Extract Injection".—These authors summarize in castrated rats, the cytological changes of seminal vesicles found after 5 to 20 days after the gonadectomy, as follows:

"We have found readily apparent cytological changes within 5 to twenty days after castration, involving regression of the secretory epithelium, loss of individual and constant elements of the cells of the normal tissue and changes in GOLGI bodies. On the basis of the cytology of the seminal vesicles, one can easily detect a ten days castrated animal; by injecting adult rats subcutaneously with testis extract, beginning immediately after operation, we have prevented the development of the typical castration changes in the seminal vesicles; animals castrated for twenty days, but uninjected, have both smaller seminal vesicles than they had at the time of operation".

"We have thus prevented the development of all the typical cytological castration changes by injection of the testis extract. Furthermore, we have produced, cytologically, normal seminal vesicles in animals that were prepubertally castrated and hence had not developed the typical normal vesicle until after being injected with the extract".

The mentioned abstracts are those connected with our own work. By reading it, we may not conclude from the mentioned work, which were the characteristics adopted for the test so as to establish a standard, viz.: age of animals, limit of duration of the injections, in the incidence of quantity to be used.

We believe mouse a most practical material for this kind of work. We preferred it, because the phenomena of regression appear earlier, and owing to its higher sensibility, being of a smaller weight, about one fifth lighter than the rat.

Some points of the present work will be further and more accurately developed in notes to be read at the next meeting of the Sociedade Brasileira de Biologia, and to be published in the Comptes Rendus de la Société de Biologie de Paris, and more extensively elsewhere.

The enclosed plates show examples of our own results.

It is a great pleasure to us that, independently of MOORE and GALLAGHER, we have been able to arrive, with mice, to results which, in general lines, are concordant with those obtained in rats by those eminent specialists.

Laboratory of Physiology, Oswaldo Cruz Institute.

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BIBLIOGRAPHY

1—STEINACH—Pflügus Arch., 56, 1894.
2—Mc GEE, JUHN and DOMM—Am. J. Phys., 87, 406.
3—MUTO—Act. med. Keijo, 11, 125, 1928.
4—MOORE—J. exp., zoology, 1, 455, 1928.
5—PEZARD—J. Physiol. Path., gen., 700, 1922.
6—LIPSCHÜTZ—The int. secret. of sex glands.