dosages of folic acid were used in the synthesis of a biologically active but microbiologically inactive compound. This view has been taken previously by Wright *et al.*[10] who observed a decrease of folic acid (as measured microbiologically) in incubated rat liver tissue. In any event, low tissue levels of folic acid are utilized efficiently on low folic acid diets. The possibility of storage in some other tissue of the animal seems improbable since analysis of the various body tissues of chicks receiving 200 γ of folic acid per 100 g of diet showed the liver to be the main site of storage.

Results from the second series also demonstrate that high initial doses of orally administered folic acid are used as efficiently as equivalent doses of injected folic acid. Group 4, which received the folic acid supplement by the oral route, gave a growth response at 3 weeks equivalent to that of the injected group (Group 3); and at 5 weeks the growth of the 2 groups plateaued at the same weight (difference of 10 g is not considered significant). These results do not substantiate the findings of Frost and Dann[11] who found differences in favor of the injected folic acid but a direct comparison of the data is impossible since different technics and animals were involved.

Liver, kidney, and pancreas were found to have the highest and skin and muscle the lowest content of folic acid; testes, spleen, heart, brain, and lung had intermediate values. The relative content of folic acid in chick liver, heart, and brain agree with the values found by Williams *et al.*[12]

Summary. High oral intakes of folic acid are stored inefficiently in chick livers and have little if any effect on the content of this vitamin in chick muscle tissue. Chicks given large oral doses of folic acid and then placed on a folic acid-deficient diet grew as well as chicks receiving an equivalent amount of folic acid by injection. The distribution of folic acid in some representative chick tissues has been studied.

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Intrasplenic Transplantation of Testes in Castrated Mice*

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Granulosa-cell tumors and luteomas have arisen in intrasplenic transplants of ovaries in castrated male and female mice.[1,2] The pathogenetic factors responsible for these ovarian tumors have been assumed to be (1) the capability of the liver to inactivate ovarian hormones, and (2) the increase in the amount of gonadotrophic hormones that

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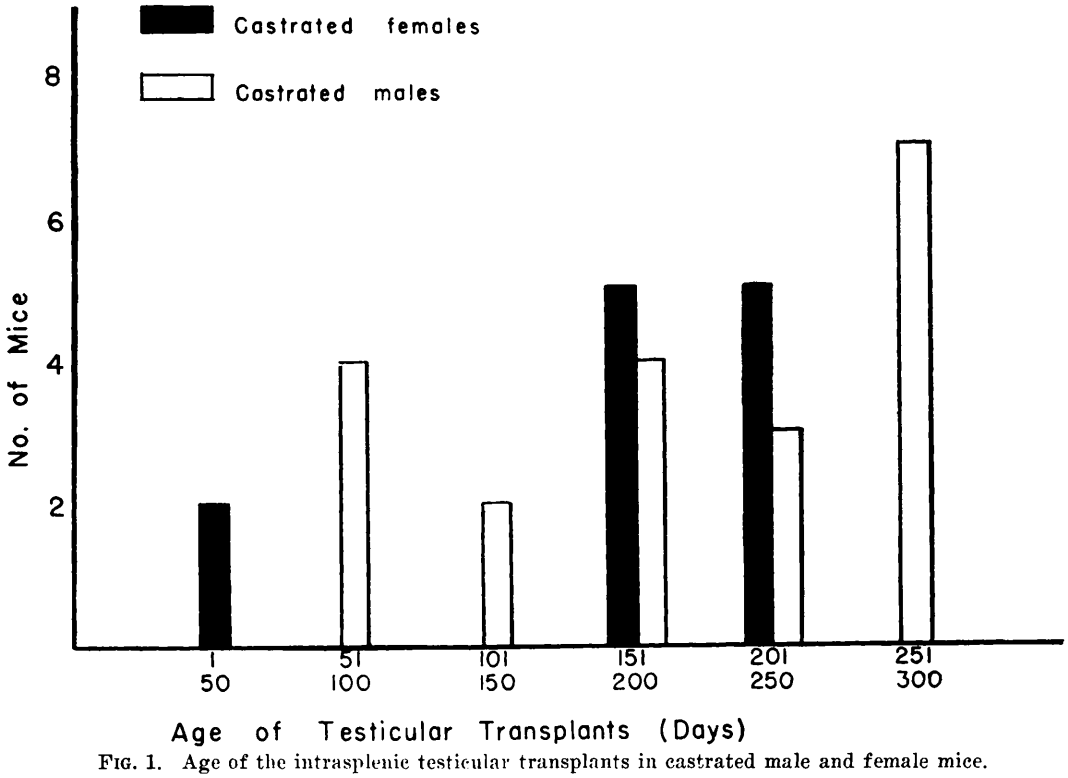

FIG. 1. Age of the intrasplenic testicular transplants in castrated male and female mice.

follows castration. A failure to obtain androgenic effects on the accessory genital organs of castrated male rats and rabbits occurred when androgens were administered so that they passed through the hepatic portal system before gaining access to the general circulation.[3-7] Biskind and Biskind[8] have reported that one granulosa cell-like tumor developed in an intrasplenic testicular graft in a castrated male rat. The fate of intrasplenic testicular transplants in castrated male and female mice and evidence for their endocrine effects are reported in this paper.

Materials and Methods. Inbred mice of the A strain were used.‡ Both male and female mice were castrated when they were 1 to 3 months of age, and a testis obtained from 2- to 7-day-old male mice of the same strain was transplanted into the spleen of each animal at the time of castration. The animals were fed on a commercial diet (Nurishmix), with occasional supplements of grain (a mixture of wheat, oats, sunflower seeds, and calf-meal pellets), and water. The age of the intrasplenic transplants of testes at the time of autopsy is given in Fig. 1.

Results. The spleen provided a good vascular supply for the testicular grafts: they increased in size shortly after the transplantation as observed at laparotomy. The transplants usually protruded from the surface of the spleen; the largest measured about 4 x 8 mm in diameter (Fig. 2).

Twenty testicular grafts were recovered from castrated male mice bearing intrasplenic transplants for a period ranging from 63 to

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‡ These mice were supplied by Doctors A. Gorbman, C. W. Hooker, and L. C. Strong.

275 days. Testicular grafts were not found, however, in 2 hosts sacrificed at 273 and 296 days after the operation. Microscopically, the testicular grafts were well incorporated with the splenic tissues and blood sinuses although major portions of the grafts were covered only by the splenic capsule. A few layers of proliferating spermatogonia and spermatocytes were observed in the seminiferous tubules of transplants recovered after 63 to 140 days. Sloughing of the spermatogenic cells into the lumina of the tubules, resulting in a disorganized appearance of the seminiferous epithelium, was frequently encountered. A slight increase in the number of interstitial cells of Leydig, as compared to a normal testis, was noted in 3 testicular grafts. Minute vacuoles were present in the cytoplasm of many of the interstitial cells. In testes grafted into the spleens for a period of over 160 days the seminiferous epithelium was generally thin, although the lumina of the seminiferous tubules remained large (Fig. 3). A few large primary spermatocytes containing 2 or more nuclei were noted in many of the tubules. The nuclei in these large primary spermatocytes were usually at the same stage of meiosis or were in a resting stage with a distinct nuclear membrane. Multinucleated masses containing pycnotic nuclei were also noted in some tubules. The interstitial cells were present in the coagulated tissue fluid that occupied the intertubular spaces (Fig. 4). In 2 grafts a few seminiferous tubules were necrotic. Fibrous and splenic tissues invaded parts of these 2 grafts. Of particular interest was the finding of spermatids in a testis 243 days subsequent to intrasplenic transplantation. A small group of spermatids, with at least 3 cells showing late spermiogenesis, and many scattered spermatids with indication of sperm head formation were found in the seminiferous tubules. A large number of secondary spermatocytes was present in this testis (Fig. 5). In all other testicular transplants, however, spermatogenesis usually stopped at the primary spermatocyte stage.

The prostates and seminal vesicles of the castrated male mice with intrasplenic testicular transplants without adhesions were atrophic. In one mouse with vascularized adhesion of the graft to the left kidney the accessory genital organs appeared normal when examined 99 days after transplantation, and the adrenal glands showed degeneration of the x-zone. Effects of castration were observed in the Bowman's capsules of the kidneys,[9,10] and in the submaxillary glands.[11,12]

Among 18 castrated female mice with intrasplenic transplants of testes, 12 testicular transplants were recovered after a period ranging from 23 to 224 days. In 2 testes that had been grafted for 23 and 38 days, spermatogenesis had not proceeded further than the primary spermatocyte stage. Normal numbers of the interstitial cells were noted in these 2 grafts. Histological features of the testes grafted for a period of over 150 days were generally similar to the corresponding transplants in the castrated male mice, except that the interstitial cells were perhaps more numerous (Fig. 6).

The uteri of the castrated female mice bearing intrasplenic testicular grafts were atrophic and weighed less than 25 mg. No x-zone was observed in the adrenal glands; androgenic effects were not noted in the kidneys and the submaxillary glands showed castration changes.

Discussion. In view of the relatively high percentage of successful transplants, the spleen appears to be a favorable site for experiments on the intraperitoneal transplantation of testes. Pfeiffer,[3] and Turner[13] reported that testicular transplants in the livers and kidneys of rats start to develop well but usually become infiltrated with fat and connective tissue and then degenerate.

Microscopical examination of the present material reveals that the intrasplenic transplants of testes in castrated male and female mice resemble, in general, the cryptorchid

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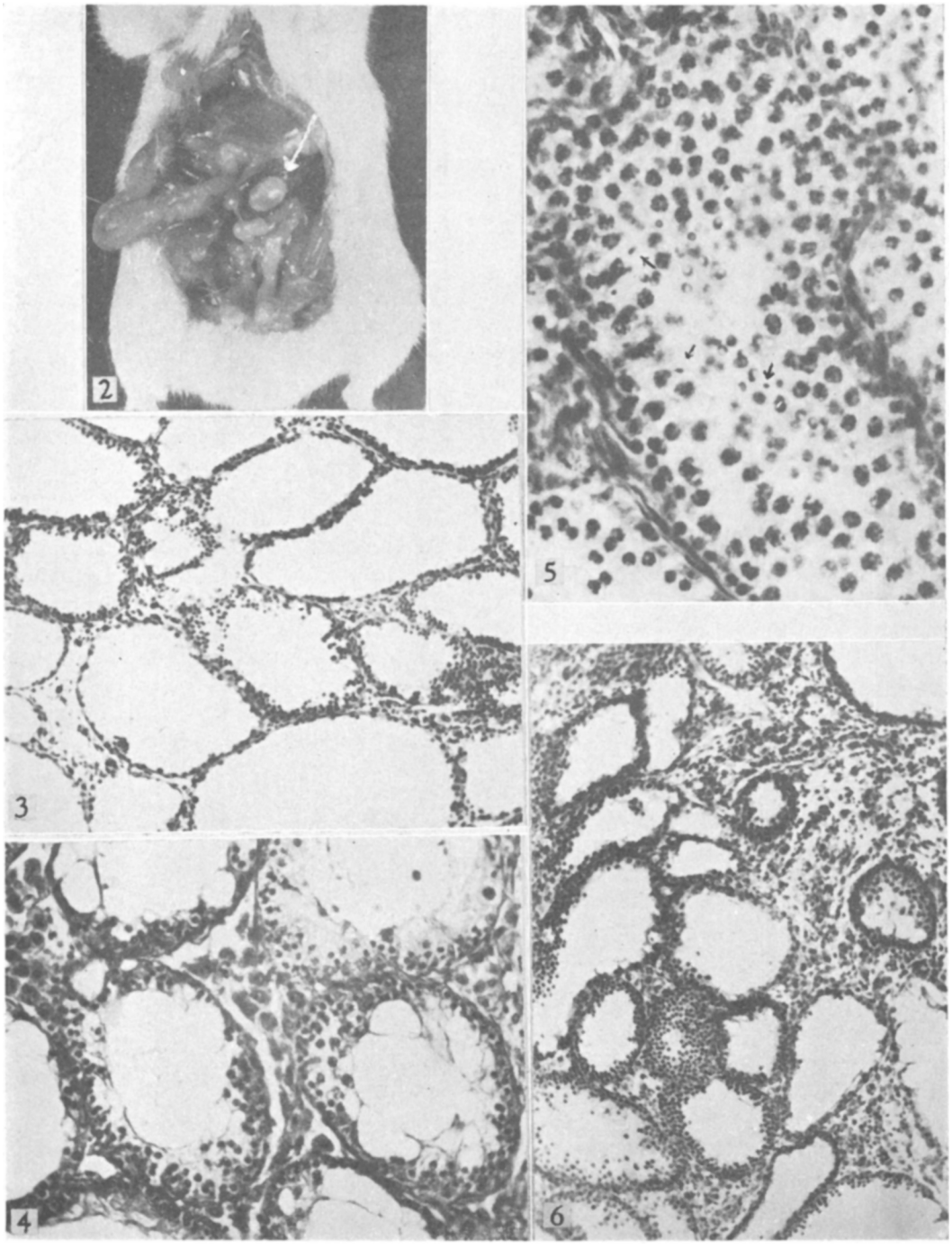

FIG. 2. Photograph of a castrated male mouse bearing an intrasplenic testicular graft for 274 days. Arrow indicates the graft. Note atrophy of the seminal vesicles and prostates.

FIG. 3. Section of a testis grafted in the spleen of a castrated male mouse for 257 days. Photomicrograph × 80 (approx.).

FIG. 4. Section of a testis grafted in the spleen of a castrated male mouse for 208 days showing the interstitial cells and disorganized seminiferous epithelium. Photomicrograph × 192 (approx.).

FIG. 5. Section of a testis grafted in the spleen of a castrated male mouse for 243 days showing spermatocytes, spermatids, and three spermiogenic cells (arrows pointed). Photomicrograph × 380 (approx.).

FIG. 6. Section of a testis grafted in the spleen of a castrated female mouse for 165 days. Note the number of the interstitial cells and thin seminiferous epithelium. Photomicrograph ×86 (approx.).

testes described in many other mammalian species.[14] That the normal spermatogenic activity in retained testes is impaired by the higher temperature in the abdominal cavity is known, but information concerning the definite mechanisms that govern the normal process of spermatogenesis is still incomplete. The observation of large primary spermatocytes containing 2 or more nuclei in the testicular grafts indicates that the cytoplasmic divisions do not proceed normally even in the spermatogonia. It is interesting to find secondary spermatocytes, spermatids, and early formation of spermatozoa in a testis transplanted into the spleen for a period of 243 days. Complete spermatogenesis in testes that presumably resided inside the abdominal cavity has been reported in 2 hermaphroditic mice by Blotevogel,[15] and Hooker and Strong.[16]

The maintenance of accessory genital organs in a castrated male mouse with vascularized adhesion of the testicular graft to the left kidney indicates that intrasplenic grafts produce androgen. The histological appearance of the interstitial cells of Leydig in the testicular transplants affords further indication of the functional activity. The castration effects found in organs of the castrated male mice bearing intrasplenic transplants of the testes suggest that the liver inactivates the androgenic hormone produced by the graft. With the mice thus physiologically castrated as far as androgen in the general circulation is concerned, it seems probable that the gonadotrophic hormones of the hypophysis are increased.[17] This would explain the functional appearance of the interstitial cells in the intrasplenic grafts and would agree with the observations of Nelson[18] and Moore[19] who reported that the hormone secretion by experimental cryptorchid testes in rats is stimulated by injections of gonadotrophic hormones. Turner[13] observed hypertrophy and hyperplasia of the interstitial cells of Leydig in intraocular transplants of testes in castrated rats, but the transplants in noncastrated rats invariably contain sparse interstitial elements.

Histological study of the present material shows no indication that the intrasplenic testicular transplants in castrated male and female mice may develop into a granulosa cell-like tumor such as reported by Biskind and Biskind[8] in a castrated male rat under similar circumstances. The failure to find tumors in the present experiment suggests that intrasplenic testicular transplants do not as readily become tumorous as do intrasplenic ovarian grafts in castrated mice.[1,2]

Summary. Thirty-two successful testicular homotransplants were recovered from 40 castrated male and female mice of the A strain bearing the intrasplenic transplants for a period ranging from 23 to 275 days. The testicular transplants showed the general histological features of cryptorchid testes, although spermatids and sperm heads were noted in one case after 243 days of transplantation. The present material gave no indication of tumor development in the intrasplenic testicular transplants. The experimental data suggested that the endogenous androgen produced by the intrasplenic testicular transplant was inactivated when passing through the hepatic portal system.

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