TECHNICAL PAPERS

Vaginal Absorption of Penicillin

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Vaginal suppositories containing 100,000 units of penicillin in a base of cocoa butter[1] were used on 20 patients divided into three groups: (1) nonpregnant women complaining of profuse leucorrhea and/or pruritus, (2) pregnant women a few days or weeks before delivery, and (3) women recently post partum. Two hundred thousand units were inserted at one time, and at least three serum levels of penicillin[2] were determined at intervals ranging from ½ to 8 hours afterward.

Group 1. Nine nonpregnant patients with vaginitis or chronic cervicitis, with a profuse discharge and/or pruritus, were treated. All patients experienced relief following penicillin treatment. Two patients had *Trichomonas* infections. In one, the organisms were still found after treatment, but, as in the other, the symptoms were absent. At least three serum levels were determined on all patients: in 5 patients, after ½ hour levels ranged from .312 to 1.250 units of penicillin/cc. of serum with an average of .687 unit; in 9 patients, after 1 hour, from .039 to 1.250 units with an average of .655 unit; in 4 patients, at 3 hours, from 0 to .156 unit with an average of .068 units; in 7 patients, at 4 hours, from 0 to .312 unit with an average of .120 unit; in 4 patients, at 6 hours, from 0 to .156 unit with an average of .039 unit; and in 3 patients, levels at 8 hours showed no concentration. As the necessary concentration for streptococcus control is considered to be .039 and for staphylococcus, from .078 to .1 unit, the critical amount was present in these patients for an average of 6 hours. There was a slightly higher rate of absorption in the patients who were near the end of their menstrual cycles and in two menopausal patients. One exception was a patient whose cycles were usually of about 28 days, and who on the 12th day had the high concentrations of .625, 1.250, .625, .312, and .156 units at ½, 1, 2, 4, and 6 hours, respectively.

Group 2. Four patients who were awaiting delivery were tested for absorption at ½, 1, and 4 hours after treatment. Of the three patients who were within two weeks of term, two showed no absorption of penicillin, and the third showed levels of .156, .039, and .078 units at the above intervals. One woman who was 2⅔ months short of term showed an absorption similar to that of nonpregnant women.

Group 3. Seven patients who had recently delivered were tested for absorption after receiving 200,000 units of penicillin. Results were similar to, even slightly better than, those in the nonpregnant patients of Group 1. Four patients, 10 days post partum, had high concentrations in their blood: at ½ hour, levels ranged from .625 to 1.250 units with an average of .938 unit; at 1 hour, from .625 to 1.250 units with an average of .781 unit; and at 4 hours, from 0 to .156 unit with an average of .063 unit. One patient, 9 days post partum, had no concentration at ½ hour and only .019 unit at 1 and 4 hours. Two patients, respectively 14 and 35 days post partum, showed moderate levels ranging from .312 to .039 unit at ½ hour, .312 to .312 unit at 1 hour, and 0 to .039 unit at 4 hours.

Summary. Except during the last two months of pregnancy, penicillin is easily absorbed from cocoa butter suppositories in the vagina, ordinarily to give therapeutic blood levels for from 4 to 6 hours. Penicillin in the dosage used seems to have a good effect on vaginal infections. In nonpregnant women, during the ovulation phase, considered as including days 14 ± 2 in the ordinary menstrual cycle of about 28 days, absorption seemed to be somewhat diminished. Higher levels were found in patients who were near the end of their menstrual cycles and in two patients who were menopausal. Patients who were very near term absorbed little or no penicillin, whereas patients 10 days post partum showed excellent absorption.

[1] Generously supplied by Schenley Laboratories, Inc.

[2] All serum levels were determined by courtesy of Dr. Chester S. Keefer in his laboratory at the Evans Memorial Hospital, Boston University Medical School, by Dr. Donald G. Anderson and Miss Marjorie Jewell, whose cooperation is gratefully acknowledged.

Tumors in Intrasplenic Ovarian Transplants in Castrated Mice[1]

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Investigations concerning the influence of steroid gonadal hormones on carcinogenesis have revealed many significant relationships. On the other hand, much less is known of the action of pituitary hormones on the development of tumors, although such relationships have been studied by injections of different pituitary preparations and by observing the effects of hypophysectomy on tumor-bearing animals or animals given carcinogenic substances (*10*). Recently, Pfeiffer and Hooker (*9*) obtained small local overgrowths of testicular interstitial cells in mice of the A strain that were given daily injections of pregnant mare's serum for several months. These growths resembled early stages in the development of the interstitial cell tumors that have been induced in estrogen-treated mice of this strain. The difficulty of obtaining purified and standardized gonadotropic hormones, and the formation of antihormones, have impeded adequate study of the long-term effects of gonadotropins.

It has been shown that many estrogenic and androgenic hormones are inactivated when circulated through the hepatic portal system (*12*). Furthermore, the intrinsic production of gonadotropins is increased subsequent to castration, as determined by urinary or hypophyseal bio-assays, or in experimental parabiosis of an intact with a castrated animal (*4*). The

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role of the intrinsic gonadotropic hormones of the pituitary gland in tumor formation could thus be studied by the transplantation of gonads into the spleen or other sites drained by the portal vein. Biskind and Biskind (*2*) reported that three ovarian and one testicular tumors developed in gonads transplanted into the spleens of castrated rats, although Lipschütz, *et al.* (*7*) observed only growth of lutein cells in guinea pigs under similar circumstances. This paper is a report of tumors in intrasplenic ovarian transplants in castrated mice.

Inbred mice of the Strong A and C_3H strains[2] and hybrid mice (AC_3, AC_5)[3] were used. Six groups of experiments were set up (Table 1). Castration and grafting were done when the mice were one to three months old, except in the group bearing intratesticular transplants, in which a few mice were operated on at 130 days of age. The donors of the ovaries for the homo-or heterotransplantations were usually younger than the recipients. All experimental animals received no treatment other than that mentioned.

growth observed in a subcutaneously transplanted ovary in a castrated female mouse. The mouse was the only one that was operated on during pregnancy.

Most of the ovarian tumors protruded from the surface of the spleen; the largest measured 5 x 8 x 9 mm. in diameter. The tumors usually had a smooth, reddish-yellow surface, and some showed few scattered hemorrhagic follicles as indicated by dark red spots. On microscopic examination, most of the tumor cells of the granulosa-cell tumors appeared indistinguishable from the granulosa cells of the normal Graafian follicle, and mitoses were frequent. The ovarian tumors usually contained small and medium-sized, somewhat distorted cavities that were sometimes filled with blood cells or a few degenerating cells, and many solid masses or granulosa-like cells. The cells of the latter structures either were arranged in delicate, sinuous, single-celled cords or, in some places, assumed a trabecular appearance. Thus, the granulosa-cell tumors were preponderantly diffuse and folliculoid in pattern, as featured by Barzilai (*1*) in human material. The luteomas were composed

TABLE 1

TUMORS IN OVARIES TRANSPLANTED TO CASTRATED MICE

Site of transplant	Hosts	No. of mice	Tumors*			
			Granulosa cell	Luteoma	Mixed	None
Spleen	Castrated males	21	5 (224–268 days) 2? (130–262 days)	0	1 (225 days)	13 (75–268 days)
Spleen	Castrated females	33	0	4 (153–284 days)	7 (266–346 days)	22 (25–334 days)
Spleen (adhesion)	Castrated females	19	0	1 (262 days)	0	18 (95–346 days)
Subcutaneous	Castrated females	25	1? (243 days)	0	0	24 (61–278 days)
Subcutaneous	Intact males	12	0	0	0	12 (141–239 days)
Subcutaneous	Castrated males	9	0	0	0	9 (64–233 days)
Testis	Intact males	25	0	0	0	25 (32–353 days)

* The age of the transplant is given in parentheses.

Five granulosa-cell tumors, two probable tumors of the same type, and one mixed tumor consisting of both granulosa and luteomatous cells were found among the 21 castrated male mice with intrasplenic ovarian transplants. Four luteomas and seven mixed tumors were found among the 33 castrated female mice that showed no adhesion of the intrasplenic ovarian graft to the left uterine horn or to the adjacent peritoneum. Among the 19 castrated female mice with vascularized adhesions of the ovarian transplant, one luteoma was observed in a mouse that had irregular estrous cycles during the latter part of the experimental period. Mice of the A strain were used in these two groups of experiments, and most of the ovarian tumors developed in castrated mice carrying the intrasplenic ovarian transplants for more than seven months. No ovarian tumors were found in other sites of transplantation except for one small granulosa cell tumor-like of lutein-like cells arranged to form numerous small, tubular or cord-like structures, each surrounded by a delicate connective tissue septum. Sinusoidal blood capillaries and pigment containing cells were observed frequently among the luteinized tumor cells in some areas. Mitoses of the luteomatous cells were rare. In the mixed tumors there were transitional areas between the granulosa-cell and luteomatous types of tumor cells, which indicated that the luteomas were derived from luteinization of the granulosa cells of the tumors. In general, the histological features of the ovarian tumors were similar to those described by previous workers in the ovaries of X-rayed mice (*3, 5, 6, 11*).

The prostates and seminal vesicles of the castrated male mice with intrasplenic ovarian transplants were atrophic. Only three castrated female mice having tumors of the intrasplenic ovarian transplants showed estrous vaginal smears, two early and one in the later part of the experiment. No estrous smears were obtained from the other eight ovariectomized mice that had tumors in the intrasplenic ovarian transplants; their uteri were small, averaging about 37 mg. A de-

[2] These mice were supplied by Dr. L. C. Strong.

[3] AC_3 are first-generation hybrid mice of the following parentage: C_3H (low-tumor) ♀ X A ♂. AC_5 are first-generation hybrid mice of the parentage: A (low-tumor) ♀ X C_3H ♂.

tailed description of the histological findings, together with the physiological effects, of the ovarian grafts and ovarian tumors induced in the present experiments will be reported elsewhere.

It is of especial interest that the pure granulosa-cell tumors and luteomas developed only in the castrated male and female mice, respectively, bearing the intrasplenic ovarian transplants. This may be due to structural and physiological differences in the pituitaries of male and female mice similar to those observed in rats (*8*).

Experimental induction of ovarian tumors in mice following X-ray irradiation has been reported previously (*5, 6, 11*), although no explanation as to the cause of the neoplastic formations was given. Because the histological structure of granulosa-cell tumors and the luteomas appearing in the ovaries transplanted into the spleen were similar, if not identical, to the ovarian tumors induced with roentgen rays, it seems that the same mechanism, namely, gonadotropic overaction, might be responsible for the tumors under both circumstances.

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Human Amino Acid Requirements

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A recent report (*2*) from Mitchell's laboratory presented data from which were calculated the requirements of normal young adult female humans for nitrogen balance for three different proteins: milk, soy flour, and white flour. From these figures and the analytical data summarized by Block and Bolling (*1*) it is possible to follow the pattern of estimation described by Stare, *et al.* (*3*) for egg protein, and calculate some of the individual amino acid requirements for humans on these bases.

For convenience of comparison, Mitchell's protein requirement figures for nitrogen balance, based on a 59.55-kg. female subject consuming a total of 1,300 calories, were extrapolated linearly to 70-kg. subjects. The corresponding data of Stare's estimate, which so far lacks experimental verification, are included in Table 1, while the calculated results for the individual amino acids are given in Table 2.

Theoretically, such calculations should have validity only if the biologic value of the protein is essentially unity. The fact that agreement is quite good, despite the wide range of biologic values reported by Mitchell (0.41–0.74), suggests that biologic value may be determined largely by the "essential" amino acid content, and the fraction of absorbed nitrogen excreted in the urine (which reduces the biologic value from unity) may merely reflect the excess of "nonessential" amino acids which cannot be utilized for lack of the necessary quantity of "essentials."

TABLE 1

Human Protein Requirements for Nitrogen Balance

Protein	Grams required for equilibrium in females (59.55 kg.) on 1,300-cal. diets (N × 6.25)	Coefficient of true digestibility	Grams protein corrected for digestibility* for 70-kg. individual (N × 6.25)
Milk†	22.4	0.94	24.7
Soy flour†	23.4	0.92	25.3
White flour†	38.7	0.97	44.1
Egg‡	—	1.00	28.0

* For the purpose of these estimates it is assumed that the effect of digestibility is the same on all amino acids. Since the digestibility figures are close to unity, the error thereby introduced is not likely to be greater than the various experimental errors in the analytical data, etc.

† Mitchell's data.

‡ Stare's estimate.

TABLE 2

Human Amino Acid Requirements for Nitrogen Balance

(Grams/day)

	Protein source				Minimal quantity
	Milk	Soy flour	White flour	Egg	
Arginine	1.2	1.5	1.7	1.8	1.2
Cystine	0.8	0.2	0.8	0.5	0.2
Histidine	0.7	0.6	1.0	0.5	0.5
Isoleucine	1.3	1.2	1.6	1.3	1.2
Leucine	3.0	1.7	5.3	3.6	1.7
Lysine	1.8	1.4	0.8	1.4	0.8
Methionine	0.5	0.5	1.3	1.4	0.5
Phenylalanine	1.5	1.4	2.4	1.4	1.4
Threonine	1.1	1.0	1.2	1.2	1.0
Tryptophane	0.5	0.4	0.4	0.4	0.4
Tyrosine	1.3	1.0	1.7	1.0	1.0
Valine	1.4	1.1	1.5	1.3	1.1

Of particular interest is the relatively good agreement of these several estimates. Most striking, perhaps, are the relatively low quantities of cystine and methionine in the case of soy flour and of lysine in the case of white flour. Bearing in mind the well-known observation that lysine seems to be the limiting amino acid in the utilization of white flour by the growing rat, it is not surprising that more of that protein is required for balance than either milk or soy flour. Table 2 also reflects this state of affairs by offering, in every other case except tryptophane, substantially more of the individual amino acids