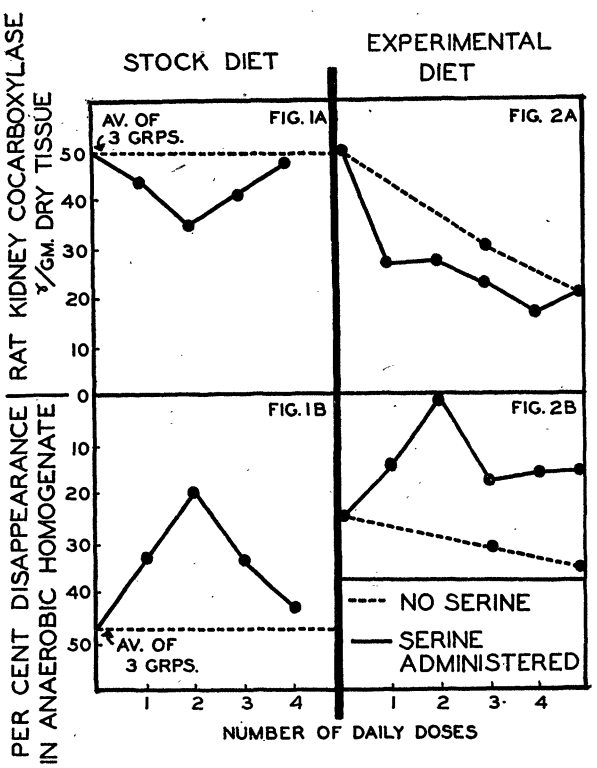

Changes in rat kidney cocarboxylase in animals receiving *dl*-serine. In figs. 1a, 1b, males (150 to 190 grams) were employed, as deaths due to serine have not been observed in rats on stock diet.[3] In figs. 2a, 2b, most of these groups consisted of smaller female rats (120 g) since on the experimental diet their mortality is less upon serine administration as compared to male rats.[3] Both these measures permitted the experiments to be completed satisfactorily. With respect to the changes in cocarboxylase, unpublished experiments have shown no sex difference.

kidney homogenates from animals on experimental diet which had received oral serine, it was possible to show more cocarboxylase in the digest than in the fresh tissue (in one case, an increase of 32 per cent.). The pyrophosphate and thiamin added to the homogenate represent the source of the extra cocarboxylase.

Discussion

In the animals receiving serine there are two phenomena evident, the reduction in kidney cocarboxylase *in vivo,* and a lessened tendency for this substance to disappear from the homogenate on anaerobic incubation *in vitro.* These biochemical events may now be correlated with the pathological findings.[5] The first dose of serine leads to profound necrotizing lesions in the kidneys of rats on either experimental or stock diets. At this time there occurs a drop in cocarboxylase and a reduction in the breakdown of cocarboxylase in anaerobic homogenate. Processes of repair are very extensive and almost completed by the fourth day in animals on the stock diet, and here we see a return to normal of the tissue cocarboxylase and of the rate of its disappearance on anaerobic incubation. In the animals on the vitamin B deficient diet, however, the kidneys never return to their former healthy state and the necrotic tissue is replaced by calcium deposits. Here we find no return to normal cocarboxylase levels or to a normal rate of its disappearance on incubation.

With reference to the reduction of kidney cocarboxylase *in vivo,* there may be several explanations, all equally worthy of consideration. Thus, the diminution in cocarboxylase may be the result of tissue damage, normal levels (Fig. 1a) returning when the tissue is repaired. Again, the observations may be ascribed to a specific involvement of cocarboxylase in serine metabolism, or perhaps to an indirect action of the amino acid on tissue respiratory systems. At present, there is no direct evidence which would permit us to select any one hypothetical mechanism in preference to the others.

In the case of the lessened tendency of cocarboxylase to disappear in homogenates from animals receiving oral serine, and where actual cocarboxylase synthesis occurs in the presence of added serine, the mechanisms involved seem even more obscure. One has the feeling, however, that these reactions involve the functioning of tissue cocarboxylase phosphatase.

Summary

When rats on either a stock or a vitamin B deficient diet are given the amino acid *dl*-serine by stomach tube, there is a fall in the cocarboxylase concentration of the kidney. This organ exhibits marked damage. There is, also, a lessened tendency for cocarboxylase to disappear from the tissue homogenate upon anaerobic incubation *in vitro.* In animals on the stock diet, only, there is a recovery of the normal cocarboxylase level. The probable relation of these findings to the mechanism of the production of the injurious action of serine is discussed.

We would like to acknowledge the helpful criticisms of Dr. C. Artom.

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CASTRATION EFFECTS OF THE INHERITED HORMONAL INFLUENCE[1, 2]

WOOLLEY, Fekete and Little[3, 4] observed evidence of

[1] A preliminary report of material being compiled for a thesis in partial fulfilment of the requirements for the degree of doctor of philosophy at the University of Minnesota.

[2] Aided by a grant from the Graduate School Cancer Research Fund made to Dr. Bittner.

[3] E. Fekete, G. Woolley and C. C. Little, *Jour. of Exp. Med.,* 74: 1–8, 1941.

hormonal stimulation in mice of the cancerous dilute brown and C3H stocks that had been castrated shortly after birth. Because of the hyperplastic changes noted in the cortex of the adrenals, it was believed that there was the source of the hormonal stimulation in the absence of the gonads. Spontaneous mammary carcinomas were found in castrated mice of both strains.

Since both the C3H or Z and the dilute brown mice used by the workers cited have a high incidence as virgins, it seemed advisable to study the influences active among castrates of two high tumor strains which differ in the incidence of mammary cancer in the breeding and the virgin state. For this purpose, female mice of the high cancerous A and C3H stocks were chosen. As breeders, mice of both of these strains show a high cancer incidence.[5, 6] As virgins, however, the A females have a low incidence[7] in contrast to the much higher percentage reported for the Z or C3H stock.[8] The variation in the incidence between the virgins of these two strains has been found to be caused by a difference in genetic factors,[9, 10] and the character has been termed the inherited hormonal influence.

In the present study the mice under observation include females of these two high tumor strains as well as reciprocal hybrids. Some of the hybrids have the active milk agent and are called high tumor hybrids; those without this agent are considered as low tumor hybrids. The mice employed were castrated at approximately four weeks of age. Vaginal smears were then made at six-week intervals to secure evidence of hormonal stimulation. There was an additional two-week period of daily observation of the vaginal response of the castrate mice and of comparable groups of normal virgin animals. A few animals within each group have been sacrificed. Whole mounts are being made of the second and third mammary glands, and are being studied to determine the relationship of their architecture to the hyperplastic areas in the adrenal cortex and the changes in the uterus. All animals were fed with Purina Fox Chow and water ad libitum.

As Woolley, Fekete and Little noticed in the Z and the dilute brown castrates,[4] we have also observed that the vaginal orifice of castrated Z mice, as well as the hybrids, was opened within two weeks following ovariectomy. Some mice were in estrus as early as five months following castration, whereas others demonstrated these changes at a later period. Since the first animal to develop a tumor was seven months of age, this age has been considered the basis for the analysis of the present data (Table 1).

TABLE 1

OBSERVATIONS UPON THE OCCURRENCE OF ESTRUS AMONG CASTRATED AND NORMAL VIRGIN FEMALES, SEVEN MONTHS OF AGE OR OLDER

Stock	Castrated females			Normal virgins
	Total number under observation	Number surviving 7 months or longer	Per cent. showing estrus at 7 months	Per cent. showing estrus at 7 months
A	79	45	0.0	75.4
Z	66	46	69.5	75.0
High tumor hybrids	133	99	79.0	90.5
Low tumor hybrids	73	73	76.7	...*

* None studied.

The mice of the A strain were strikingly different. They rarely showed an opening of the vaginal pore until at least six months after castration and no estrus cycles have as yet been observed among the castrates.

Among the mice which have been sacrificed, the Z mice and the hybrids, including those without the milk agent, have enlarged cystic uteri well supplied with blood vessels. There was gross and microscopic hyperplasia of the adrenal cortex. The uteri of the mice of the A strain were all small with no evidence of stimulation. The adrenals were also normal in their gross appearance and only two animals showed beginning hyperplasia. A complete analysis of the histological details will be presented in another report.

The mammary glands of all the Z and the hybrid mice showed extensive duct development and indications of active growth in the end bulbs. There was very slight lateral branch expansion. Precancerous nodules were observed as was spontaneous mammary cancer in mice of the Z stock and the hybrids with the milk agent (Table 2). Although the hybrids without the milk agent showed adrenal changes and mammary gland development comparable to the high tumor animals, no precancerous nodules have been observed and it is expected that no mammary tumors will develop.

[4] G. W. Woolley, E. Fekete and C. C. Little, *Proc. Soc. Exp. Biol. and Med.*, 45: 796–798, 1940.
[5] J. J. Bittner, *Canc. Res.*, 3: 441–447, 1943.
[6] *Idem, Canc. Res.*, 4: 159–167, 1944.
[7] *Idem, Pub. Health Rep.*, 54: 1113–1118, 1939.
[8] M. B. Visscher, Z. B. Ball, R. H. Barnes and I. Sivertsen, *Surgery*, 11: 48–55, 1942.
[9] J. J. Bittner, R. Huseby, M. B. Visscher, Z. B. Ball and F. Smith, SCIENCE, 99: 83–85, 1944.
[10] W. E. Heston and H. B. Andervont, *Jour. Nat. Canc. Inst.*, 4: 403–409, 1944.

TABLE 2

OBSERVATIONS OF TUMOR DEVELOPMENT, ADRENAL AND MAMMARY HYPERPLASIA IN CASTRATED FEMALES

Stock	Number sacrificed	Number of tumors	Adrenal hyperplasia	Mammary nodules
A	13	0	slight	absent
Z	4	4	present	present
High tumor hybrids .	27	15	present	present
Low tumor hybrids .	11	0	present	absent

The animals of the A strain showed great individual variation in the extent of growth of their mammary glands. No precancerous lesions have been detected.

It is apparent that there is a relationship between the incidence of breast tumors in mice and the degree of hormonal stimulation. The development of mammary cancer in virgin females of cancerous strains has been demonstrated to be partially dependent upon an inherited character. This inherited hormonal influence may also be responsible for the adrenal cortical hyperplasia in castrated mice. The adrenal changes are apparently followed by hormonal stimulation of the uterus resulting in estrus, mammary gland development with precancerous nodules, and eventually the appearance of mammary tumors in those animals with the active milk agent. In the castrated animals without the active milk agent there were adrenal changes but precancerous lesions and mammary tumors have not appeared. Castrated animals having the milk agent but not the inherited hormonal influence may show modified adrenal changes but no other evidence of hormonal stimulation.

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QUININE ACTION IN BACTERIAL GROWTH AND DISINFECTION[1]

RECENT studies of the concentration, temperature and pressure relationships of quinine action on bacterial luminescence have indicated that the drug promotes a reversible denaturation of the protein catalyst concerned in light emission.[2] The effects of quinine on dehydrogenase activity in washed cells of *E. coli*, in relation to drug concentration, temperature and presence of bacterial extracts and coenzyme, have given no clear evidence of a protein-denaturing action of quinine on these systems except at relatively high temperatures.[3] Slight inhibitions of dehydrogenase activity, observed throughout the low temperature range, appeared to result from the formation of a loose complex between the drug and a coenzyme. Both types of effects, *i.e.*, in relation to protein and to coenzyme, are evidently involved in the action of quinine on growth rates and viability of bacteria, as indicated herein.

With *E. coli* as a test organism, growing in a glucose-asparagine "synthetic" medium,[4] the addition of quinine causes an immediate slowing of growth or a disinfection, depending on a narrow range of concentration added (Fig. 1). The bacteriostatic or disinfecting action ceases at once on dilution of the medium, and growth is resumed at approximately the rate of the control (Table 1).

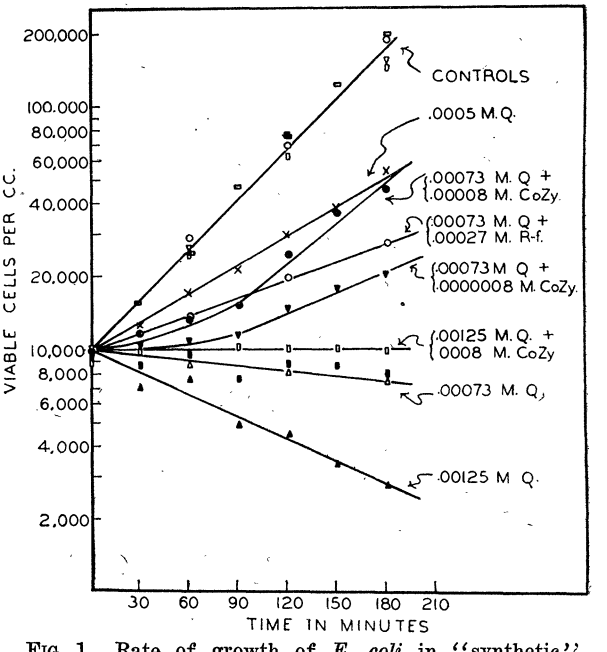

FIG. 1. Rate of growth of *E. coli* in "synthetic" medium at 37° C in relation to quinine (Q), cozymase (CoZy) and riboflavine (R.f.). Results of four experiments. Some of the curves have been extrapolated a short distance on the ordinate to coincide with 10,000 viable cells per cc at zero time in each case. No increase in rate of growth of controls without quinine was caused by these concentrations of cozymase and riboflavine, respectively.

Antagonism of the growth-inhibiting and the disinfecting effects may be brought about either by the addition of pure riboflavine or of partially pure cozymase[5] preparations. The latter are more potent and the amount needed depends upon the amount of quinine present (Table 2). To antagonize the disinfection, a ratio of roughly one molecule of coenzyme to between one and four molecules of quinine is re-

[1] This study is carried on with the aid of a grant from the Cinchona Products Institute, Inc.

[2] F. H. Johnson and L. Schneyer, *Amer. Jour. Trop. Med.*, 24: 163, 1944.

[3] The results of this investigation will be presented in a subsequent publication.

[4] The medium consisted of: 0.2 per cent. glucose, 0.2 per cent. asparagine, 0.47 per cent. $(NH_4)_2SO_4$, 0.5 per cent. NaCl, 0.27 per cent. KH_2PO_4, trace of $FeCl_2$, $CaCl_2$, and $MgCl_2$, plus NaOH to make pH equal to 7.15.

[5] Preparations of cozymase, about 50 per cent. pure, were kindly supplied by Dr. F. Schlenk. Although impurities may be concerned, the results shown in Fig. 1 and Table 2 are given with respect to the amount of cozymase added.