indicate that shrews as a group may have a higher basal metabolism than other mammals. Further, since *Sorex* represents an extreme in size, it is entirely possible that it will deviate from the function which relates other mammals.

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The Milk Factor in Blood

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Bittner's (*1*) discovery that a mammary tumor-inciting agent is present in the milk of high tumor strain mice ushered in a series of researches aimed at the eventual isolation and characterization of this extrachromosomal factor. As a preliminary to isolation experiments we undertook the investigation of mouse blood with special attention to the serum component.

TABLE 1*

Fraction tested	No. of mice	No. of tumors	
Whole blood	20	1	5% ± 4.87%. Age, 11.8 mos.
Washed red cells	13	0	
Hemolyzed and dehydrated red cells	4	0	
Whole serum	47	0	
Fat-free whole serum	16	0	
Serum globulins	83	0	
Serum albumins	21	0	
Neutral fats	14	0	
Total	218	1	Mean age at death of 217 tumor-free mice, 24.4 ± .07 mos. S.D., 1.18 ± .05 mos.

* Only animals surviving six months or more appear in this table.

Woolley (*3, 4*) had found the agent to be present in the whole blood of his strains, in concentrations suggestively similar to its concentration in milk.

In our experiments whole blood was obtained by heart puncture from 100 mature etherized female Paris mice, whose tumor incidence in our laboratory has been 92.9 ± 1.84 per cent in bred female controls. All blood samples were pooled, and serum and serum fractions were prepared in the appropriate manner from this pool. Single doses of 0.2 cc. of whole blood or serum, or serum fraction equivalent to 0.2 cc. serum, were injected subcutaneously into young female C57 test mice, a strain in which there have been no spontaneous mammary carcinomata during the past five years in our laboratory. This strain has proved highly susceptible to the milk factor, developing 76.1 ± 4.34 per cent carcinoma of the breast in females by foster nursing (*2*). The injected mice were subsequently bred and allowed to bring up their young normally under our standard control conditions. The final results appear in Table 1.

The fact that no tumors appeared in the series treated with whole serum is a definite negative answer to any hope of using serum for the isolation of the agent. All serum fractions gave results consistent with those of whole serum. The appearance of one tumor in 20 mice treated with whole blood is probably significant, but the incidence shown is less than that obtained by Woolley with whole blood.

Whole blood and blood fractions do not appear to be a rich source of the milk factor.

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Colistatin: A New Antibiotic Substance With Chemotherapeutic Activity

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Among antibiotic substances inhibiting growth of gram-negative bacteria, streptomycin, produced by *Streptomyces griseus* (*6*), appears to be particularly interesting at the present time. It was observed by us that some strains of aerobic sporulating bacilli isolated from soil, while growing upon the surface of nutrient agar containing tryptone and glucose, produce a well-diffusible antibiotic substance inhibiting growth of staphylococci as well as of *Bacterium coli*. While growing upon the liquid medium, these bacteria form heavy surface pellicles, and the antibiotic diffuses into the nutrient broth. However, this antibiotic substance is thermolabile and is strongly inactivated by boiling the culture liquid in the water bath for 15 minutes.

After detailed examination of a large number of cultures isolated from chernozem soils, an aerobic sporulating bacillus was found by us which produces a thermostable antibiotic substance, inhibiting the growth of *Staphylococcus aureus* as well as of *B. coli*. The activity of this substance is not decreased by boiling the culture fluid for 15 minutes. Because of its

bacteriostatic action upon *B. coli*, this substance may be designated as *colistatin*.

Bacteria producing colistatin in a tryptone-glucose medium also form a bright yellow pigment which is devoid of antibiotic action. When the bacteria are grown in a shallow layer of nutritive liquid for three days at 28° C. and the culture fluid is poured off (free of bacterial pellicle) and acidified by HCl to pH 3.5, a flaky precipitate is immediately formed, whereas all the activity remains in the clear filtrate. Hence, colistatin differs essentially from tyrothricin (*2*) and gramicidin S (*3*), which are precipitated by acid. When a clear filtrate containing colistatin is treated by 0.5 per cent charcoal at acid reaction, the yellow pigment is adsorbed upon the carbon, whereas the active antibiotic substance remains in the colorless filtrate. Colistatin cannot be extracted from the culture liquid by butanol, and in this respect it evidently differs from bacitracin (*4*).

Colistatin inhibits staphylococci more strongly than *B. coli*. Taking the activity against *Staph. aureus* as a standard, the unit of colistatin may be defined as that amount of the substance which is just sufficient to inhibit completely the growth of staphylococci in 1 ml. of nutritive broth for 20 hours at 28° C. When bacteria producing colistatin are grown upon tryptone-glucose medium, on the third day of growth the concentration of colistatin attains 200 units/ml. of the culture fluid. On the mineral medium, with 1 per cent of glucose, its concentration attains 1,000 units, and with 3 per cent of glucose, correspondingly, 2,500 units/ml. Colistatin is equally effective in agar or broth; its activity is not affected by the addition of 10 per cent of human blood serum to the nutritive medium; and while dissolved in agar, it rapidly diffuses into water.

For the production of colistatin the following medium is used: glucose, 30 grams; $NaNO_3$, 3 grams; KH_2PO_4, 1.5 grams; $MgSO_4$, 0.5 gram; $FeSO_4$, 15 mg.; tryptone, 1 gram; tap water, 1 liter; pH 6.5; and growth for 3 days at 28° C. in shallow layers of medium. At 37° C. the production of colistatin is decreased, and its isolation from the culture fluid by the methods outlined below cannot be attained.

It was recorded that colistatin possesses some properties in common with streptomycin and can be isolated and purified in the following manner: Culture liquid, free of bacterial pellicle, is acidified to pH 3.5, treated by 0.5 per cent of charcoal at acid reaction (colistatin is not adsorbed upon carbon in acid medium), filtered, neutralized, and treated by 1.5 per cent of charcoal at neutrality. Colistatin is completely adsorbed on charcoal at neutral reaction and can be eluted from it by 80 per cent aqueous ethanol at acid reaction (pH 5.0). The eluate is neutralized, and ethanol removed *in vacuo* at 50° C. The remaining liquid represents a concentrated aqueous solution of partially purified colistatin. It is clear that the method of concentration and partial purification of colistatin employed by us is reminiscent of the preparation of streptomycin (*1*).

A further study of the chemical properties of colistatin also revealed in its behavior some features in common with streptomycin. When a concentrated aqueous solution of colistatin is evaporated *in vacuo* just to dryness, the residue appears to be quite soluble in acid methanol, less so in acid ethanol, and insoluble in butanol. From the acid methanol solution, colistatin is precipitated by ether. However, these manipulations are associated with a partial loss of the activity of colistatin.

Colistatin can easily be obtained in the active form. After pretreatment of the culture fluid by charcoal at an acid pH, and adsorption of colistatin upon neutral charcoal, the latter is shaken energetically for 15 minutes in 4 volumes of acid methanol (containing 0.5 ml. of HCl/100 ml. of methanol). This treatment elutes colistatin from the carbon. The eluate is immediately neutralized by sodium bicarbonate to pH 6.5, concentrated *in vacuo* to one-tenth of the original volume, and filtered through a thin layer of kaolin. Colistatin, which is now contained in the filtrate, can be precipitated from the latter by 7 volumes of acetone. As has been shown, the same procedure can be employed satisfactorily in the preparation of streptomycin (*5*).

A concentrated aqueous solution of colistatin is not toxic for white mice by subcutaneous, intramuscular, or intravenous injections at a concentration of 100,000 units/kg. The chemotherapeutic properties of colistatin were tried on mice infected by the spirochete of relapsing fever (*Borrelia sogdianum*). On the day following infection, one-half of the mice received three intramuscular doses of colistatin (30,000 units/kg. in each dose, distributed at three-hour intervals). On the day following treatment, the blood of all control mice contained spirochetes, which were totally absent in the blood of all mice treated with colistatin.

In contradistinction to streptomycin, colistatin possesses purely bacteriostatic action and is devoid of bactericidal power. Colistatin inhibits the growth of staphylococci, pneumococci, *B. coli, B. proteus, B. paratyphosus B., B. dysenteriae Shiga,* and *B. typhi abdominalis.*

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