various combinations, points to a similarity between the chick-growth response on antibiotics and surface-active agents. Preliminary investigations of a possible synergistic effect between surfactants and B_{12}–antibiotic supplements have been negative. Further studies on this discovery are being continued by the Nutritional Group of National Distillers Research Division.

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Hereditary Differences in Ability to Conceive Following Coitus in Mice[1]

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Recent studies of the effects on embryonic development of cortisone injected into pregnant mice included observations on the strain differences in response to treatment (*1*). The mice were of 5 genetically different stocks: strains A, C57 black, and Black and tan, all originally received from the Jackson Laboratory at Bar Harbor, a stock (N) carrying the mutant "Naked" which was inbred in this laboratory for 11 generations, and a genetically heterogeneous stock (H). Very early in the cortisone studies it was found that these 5 stocks of mice fell into two distinct groups with respect to incidence of cleft palate in the offspring of pregnant mice injected with cortisone. Stocks A and N showed a very high incidence, whereas stocks C57, Black and tan, and H produced a relatively low incidence of offspring with cleft palate.

Strain differences have also been found in the incidence of pregnancy following coitus. When a female was found with a vaginal plug it was assumed that she had been inseminated within the preceding 24-hr period.

Table 1 shows that pregnancy does not necessarily follow insemination of the adult female mouse and suggests that female mice of some stocks (A, N) are less likely to become pregnant following coitus than female mice of other stocks (C57, Black and tan, H).

TABLE 1

INCIDENCE OF PREGNANCY FOLLOWING OBSERVATION OF VAGINAL PLUG IN 5 STOCKS OF MICE

Stock	No. females with vaginal plug	No. pregnant	Percentage pregnant
A	35	5	14.3
N	14	4	28.6
C57	18	14	77.8
Black and tan	15	9	60.0
H	2	2	100.0

[1] This work is part of a project made possible by a grant from the National Research Council of Canada. Thanks are due F. Clarke Fraser for closely supervising the entire project.

TABLE 2

INCIDENCE OF PREGNANCY FOLLOWING OBSERVATION OF VAGINAL PLUG IN 2 GROUPS OF MICE*

Stock	No. females with vaginal plug	No. pregnant	Percentage pregnant
A, N	49	9	18.4
C57, Black and tan, H	35	25	71.4

* The difference between the two groups is highly significant at the 1% level ($\chi^2 = 9.8263$, $P \ll 0.01$).

In Table 2 the data presented in Table 1 are grouped according to susceptibility to cortisone treatment as measured by the incidence of cleft palate in the offspring of cortisone-treated pregnant females.

The animals of stocks A and N (both stocks highly susceptible to cortisone treatment) were significantly less likely to become pregnant following coitus than animals of C57, Black and tan, and H (the three stocks constituting the cortisone-resistant groups).

Reference

1. FRASER, F. C., and FAINSTAT, T. D. *Pediatrics* (in press).

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A Simple Technique for Repeated Collection of Blood Samples from Guinea Pigs

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In studies on the action of antibiotics, chemotherapeutics, or antigenic substances, it is frequently necessary to bleed a guinea pig at certain intervals for micro- or semimicrochemical work related to their absorption, blood concentration, therapeutic activity, etc. Frequent bleedings may also be necessary in studies on blood circulation of bacteria, viruses, or antibodies. Heart puncture is not advised when re-

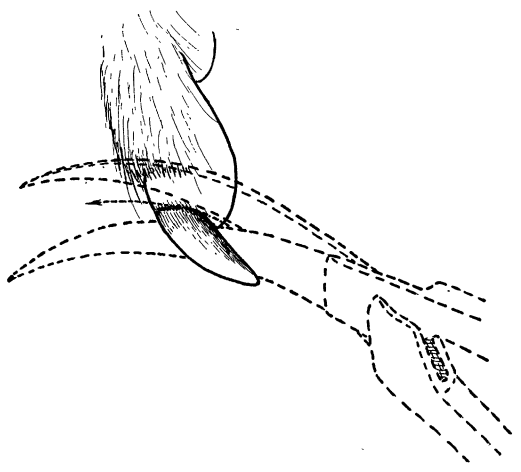

FIG. 1.

peated bleeding is necessary because of traumatic effects and the technical difficulty of obtaining repeated blood samples through this route. Bleeding from small vessels such as those of the ear lobe is difficult and not satisfactory when several blood samples are required within a short period of time.

The technique described here has been found simple and reliable in accomplishing this work. The procedure used in this laboratory allows us to draw easily small amounts of blood up to 14 times from the same guinea pig in one day. Although our experience has been limited, we believe the procedure may be used on rats as well. It has not been tried on other species.

Equipment

1 250-w, infrared ray lamp.

3% Sodium citrate solution.

Microscope slides with one or two concavities. The slide and capillary pipettes may be dry or may have been moistened with the sodium citrate solution with further drying.

Capillary tip pipettes.

Curved-on-flat dissecting scissors, 115 mm long.

Small metal spatula.

The animal's foot is cleaned to remove all interfering dirt, rinsed with the sodium citrate solution, and thoroughly dried with cotton or gauze. The nail is cut just at its insertion, giving the scissors an inclined position (Fig. 1). The foot is placed about 15 cm from the infrared ray lamp for 10 sec, which is enough to provoke dilatation of the vessels and to facilitate hemorrhage. Well-fed animals can be bled even without this irradiation. In certain infections or other pathological states, however, this detail is important, since sometimes spontaneous hemorrhage cannot be obtained.

Two bleedings can be made from the same nail insertion if a little tissue is removed from the insertion on the first cut. Thus it is rather easy to obtain up to 28 bleedings from the same animal within a short time. The blood is allowed to run from the nail to the slide concavities. Blood can be collected with a capillary pipette from the slide or directly from the cut. Bleeding is stopped by cauterizing with the small metal spatula or similar device.

The Free Energy of Hydrolysis of *p*-Nitrophenyl Phosphate[1]

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"Phosphotransferase" activity, which is found associated with certain phosphatase preparations, involves the transfer of phosphate from certain donor phosphates to suitable acceptors. We have previously reported that with some acid phosphatases of plant and animal origin, *p*-nitrophenyl phosphate can serve as a donor (*1*). Meyerhof and Green (*2, 3*) have more recently disclosed that the alkaline phosphatase prepared from intestinal mucosa also possesses phosphotransferase activity and can utilize as donors phosphocreatine, glucose-1-phosphate, and phosphoenolpyruvate as well as *p*-nitrophenyl phosphate. We have not been able to demonstrate any evidence of transfer with these first three substrates when employing citrus or alfalfa phosphotransferase. Of these substrates, only phosphoenolpyruvate was significantly hydrolyzed, and that but slightly.

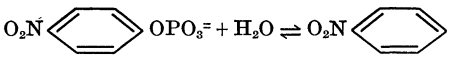
Meyerhof and Green suggest that a correlation exists between the effectiveness of a donor with the $-\Delta F^\circ$ of its phosphate bond, in the case of the three nonaryl esters. They found nitrophenyl phosphate to be an excellent donor, and if their suggestion is correct this compound should have a high free energy of hydrolysis. It is the object of this paper to report the determination of the $-\Delta F^\circ$ of hydrolysis of nitrophenyl phosphate.

The determination was made by measuring the equilibrium constant of the hydrolysis of nitrophenyl phosphate. With even a moderately low value of $-\Delta F^\circ$, the equilibrium concentration of nitrophenyl phosphate would be so low as to evade measurement by ordinary phosphate determinations. However, with P^{32}-labeled phosphate it becomes relatively easy to measure the nitrophenyl phosphate formed, after isolating it by carrier nitrophenyl phosphate.

Reaction mixture A: This consisted of 65 ml of an aqueous solution, 0.332 *M* with respect to *p*-nitrophenol, 0.123 *M* with respect to P^{32}-labeled K_2HPO_4, and containing 40 mg of a commercial alkaline phosphatase prepared by the method of Schmidt and Thannhauser (*4*). The pH was 8.95. *Reaction mixture B:* This was the same as A, except that the enzyme was omitted. Reaction time, 72 hr; temperature, 38° C.

The synthesized ester was isolated along with added carrier (0.500 g disodium *p*-nitrophenyl phosphate · 2 H_2O) after first removing inorganic phosphate as $Ba_3(PO_4)_2$. The ester was obtained by barium precipitation with 5 volumes of ethanol. Three crystallizations were sufficient to give constant activity.

The equilibrium constant was calculated for the hydrolysis written in the following way:



and it is expressed in terms of total forms of each substance (without regard to ionic form) and its dissociation constant.

$$K = \frac{(P_t)\ (A_t)}{(E_t)\ (H_2O)} \times \frac{(H^+)}{(H^+) + K_A} \frac{\frac{(H^+)^2}{k'_B\, k''_E} + \frac{(H^+)}{k''_E} + 1}{\frac{(H^+)^2}{k'_P\, k''_P} + \frac{(H^+)}{k''_P} + 1},$$

where P_t refers to the total concentration of phosphate, and k'_P and k''_P to the first and second ioniza-

[1] Enzyme Research Division Contribution No. 137.

[2] The author is greatly indebted to H. Borsook, of the California Institute of Technology, for a very helpful discussion concerning the thermodynamic aspects of this paper.