

pelage, for 29 out of 30 black rats, irrespective of diet, showed a symmetrical graying of the ingrowing fur, after 12 days of supplementation. The gray pattern outlined the lateral aspect of the head and trunk and in most cases extended from the tip of the nose to the base of the tail (Fig. 1). A graying or rusting of a triangular or diamond-shaped area on the top of the head was observed in several cases (Fig. 2). It is of interest to note that the distribution of the pattern is the reverse of that observed in rats deprived of, or receiving sub-optimal intakes of pantothenic acid. The graying persisted to some extent even after 5 weeks of therapy and was not infrequently accompanied by rusting.

Summary. It is possible to reduce materially the time necessary to produce in the rat a fully developed syndrome of biotin deficiency by feeding a diet containing in addition to 15% dried egg-white and 15% casein, 2% dried liver. On this diet most of the signs develop within less than 4 weeks.

Biotin depleted animals fed small doses of biotin curatively showed a characteristic, symmetrical lateral graying of the pelage that persisted to some extent even following 5 weeks of biotin supplementation.

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The Proteus X Bacilli and the Weil-Felix Reaction.

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The probable existence of typhus fever of the endemic type in Puerto Rico^{1,2} has recently aroused much interest among the medical profession concerning the reliability of the Weil-Felix reaction, under local conditions, in the diagnosis of this disease.

During the course of an investigation of the proteus group of bacilli, 72 cultures of proteus X organisms utilized in the diagnosis of certain rickettsial diseases, kindly supplied to us by 30 Public Health laboratories in the United States, have been studied.

Sixty-seven out of the 72 cultures investigated gave typical biological and agglutination reactions, but 5 cultures reacted atypically in one way or another.

The carbohydrates (20% aqueous solutions) were sterilized by filtration and added to the sterile medium (Difco's phenol red broth base) to obtain a final concentration of approximately 1%. For the production of

H₂S, indol and gelatin liquefaction, lead acetate agar, tryptophane broth and nutrient gelatin (all Difco products) respectively were used. Beef extract broth to which methylene blue was added to give a final concentration of 1:5000 was utilized in testing for the reduction of this dye. Rabbits were immunized by intravenous injection of fresh living suspensions.

Fermentation and other reactions, except indol, were read after 18 hr, 48 hr and one week. The production of indol was tested for after 48 hr. Alcohol-treated suspensions were used in all the agglutination tests.³

No difference was noted in the action on gelatin among the different cultures of X type bacilli studied. It was interesting to note the complete absence of agglutination, with a known typhus serum of high titer, of 21 out of the 23 proteus X-2 cultures, and the slight agglutination of 3 out of the 14 proteus XK cultures tested.

All the X-2 and X-19 cultures fermented saccharose, maltose, dextrose, salicin and galactose with the production of acid and gas and xylose and glycerol with the production

¹ Pons, J. A., *Bol. Asoc. Méd. de P. R.*, 1940, **32**, 196.

² Riera López, S., Watt, J., and Doull, J. A., *P. R. Jour. Pub. Health and Trop. Med.*, 1942, **17**, 216.

TABLE I.
Agglutination Reactions with Alcohol-Treated Suspensions of Proteus X Organisms (X-19, OX-19, X-2 and OXK) with Sera of Normal Individuals and of Hospital Patients Suffering of Conditions Other Than Typhus.

Antigen	Type of cases	Total No. of cases	1:25	1:50	1:100	1:200	1:400	Negative %
OXK	Normal Americans*	315	76.2†	18.2	1.2	0.6	0	23.8
	Normal Puerto Ricans	211	81.8	19.8	2.83	0.5	0	18.2
	Patients†	268	81.3	26.8	5.9	2.2	0.7	18.0
X-2	Normal Americans	344	50.2	27.1	8.3	3.1	0.9	49.7
	Normal Puerto Ricans	211	60.2	28.0	11.9	3.8	2.4	39.8
	Patients	259	63.9	40.1	20.8	9.3	0.8	35.8
OX-19	Normal Americans	413	45.0	22.4	9.6	1.7	0.5	55.9
	Normal Puerto Ricans	266	39.0	20.0	5.7	0.4	0	60.1
	Patients	265	42.5	21.8	6.4	2.6	1.1	57.3
X-19	Normal Americans	414	87.2	73.2	49.1	28.4	14.2	13.7
	Normal Puerto Ricans	263	84.4	66.8	45.9	22.8	8.0	15.6
	Patients	372	79.8	66.4	42.5	21.2	8.4	20.2

*Soldiers, sailors and civilians coming to the blood bank at the School of Tropical Medicine in San Juan.

†Hospital and clinic patients (Puerto Ricans) of the University Hospital in San Juan.

‡Figures refer to percentage of cases giving agglutination with the corresponding serum dilution.

of acid and gas or acid alone. Mannite, lactose, sorbitol, dulcitol, raffinose and dextrin were not fermented. The action on levulose was variable. Methylene blue was reduced; hydrogen sulfide and indol were produced.

The XK cultures did not ferment either maltose or salicin and did not produce indol; otherwise they behaved as the X-2 and X-19 cultures.

Among the atypical cultures, 3 gave atypical biological reactions but retained their typical agglutinating properties. One of the atypical cultures (supposedly X-19) did not agglutinate with the rabbit antisera (anti-X-19, anti-X-2, anti-XK) or with known typhus sera, but gave the typical biological reactions. Another culture fermented salicin and maltose but did not form indol.

Approximately one thousand persons, 700 normal and 300 clinic or hospital patients, suffering from conditions other than typhus, were tested for the presence of agglutinins in their blood against alcohol-treated suspensions³ of proteus OXK, X-2, OX-19, and X-19. The results are summarized in Table I.

³ Wadsworth, A. B., *Standard Methods*, p. 518, The Williams and Wilkins Company, Baltimore, 1939.

It is interesting to note the large number of sera which agglutinated proteus OXK in 1:25 dilution and the marked difference in the proportion of sera containing agglutinins against OX-19 as compared with X-19 antigen, especially in the higher dilutions. The fact that the sera of many persons, normal and patients alike, agglutinated the X-2 antigen must be also noted.

It is interesting to observe that there was no appreciable difference in the proportion of cases whose sera agglutinated the different antigens, among the normal Americans, normal Puerto Ricans and Puerto Rican patients.

Summary and Conclusions. 1. Out of the 72 proteus X cultures studied, 5 gave atypical reactions. One of the atypical cultures, presumably X-19, gave the typical biological reactions, but failed to agglutinate with high titer typhus serum and with anti-OX-19, X-2, X-19 and OXK rabbit-immune serum.

2. The non-liquefaction of gelatin cannot be used as a criterion in the differentiation of members of proteus X bacilli.

3. One of the atypical X-19 cultures (No. 68) has lost the power to form indol but has retained the ability to ferment maltose and salicin and to agglutinate with anti-X-19

rabbit serum and typhus serum.

4. A positive agglutination in 1:400 serum dilution is significant, when the OX-19 strain supplied by the National Institute of Health is utilized. The X-19 strain must not be used even when the suspensions are treated with phenol and with alcohol, because it gives apparently non-specific agglutination in relatively high dilution with the sera of many healthy persons.

5. The serum of a large proportion of persons agglutinated proteus OXK organisms

in 1:25 dilution. It was observed that the agglutination was strong, usually 3 plus, in 1:25 dilution and completely negative or very weak in 1:50 dilution in the majority of the sera.

6. The vast majority of the X-2 cultures tested (20 out of 23) failed to agglutinate with high titer typhus serum. This is not in accordance with the statement frequently encountered in the literature and in textbooks that X-2 organisms are agglutinated by typhus serum.

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Influence of Fat Mobilization on Acetone Body Production.

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The administration of estrogens to male or female birds results in a rapid increase in the blood lipids,¹⁻³ which is associated with the infiltration of large amounts of fat into the liver. When the administration of the estrogens is discontinued, there is a rapid return of the blood lipids to the normal concentration.

In view of these facts, it became of interest to study the influence of the rapid mobilization of fat on the rate of ketogenesis. Two groups of ducks were fasted for 3 days, and then one group was given daily a subcutaneous injection of 4 mg diethyl stilbesterol suspended in sesame oil, while the other group, used for control purposes, received a daily injection of 0.8 cc peanut oil. Every third day thereafter, until the 18th day after the beginning of the fast, blood samples were drawn for the determination of the concen-

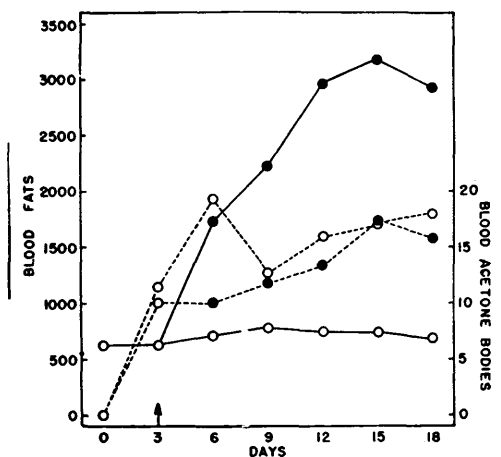


FIG. 1.

The influence of stilbesterol on blood total lipids and total acetone bodies. The open circles refer to animals receiving peanut oil and the black circles refer to animals receiving stilbesterol. The injections began on the third day after the beginning of the fast. The dotted lines depict the blood total acetone bodies, the solid lines the blood total lipids.

tration of fat⁴ and total acetone bodies.⁵

The results are depicted in Fig. 1, where each point represents the average findings from 6 ducks. Although a rapid lipemia ensued in consequence of the administration of stilbesterol, there was no significant effect on the rate of acetone body accumulation in

¹ Zondek, B., and Marx, L., *Nature*, London, 1939, **143**, 378.

² Lorenz, F. W., Chaikoff, I. L., and Entenman, C., *J. Biol. Chem.*, 1938, **126**, 763.

³ Flock, E. V., and Bollman, J. L., *Proc. Staff Meetings of the Mayo Clinic*, 1941, **16**, 783.

⁴ Street, H. R., *J. Biol. Chem.*, 1936, **116**, 25.

⁵ Mirsky, I. A., Nelson, N., and Grayman, I., *J. Biol. Chem.*, 1939, **130**, 179.