were compensated for in the following periods. The values given in Table I correspond to the 15 days comprised between the 25th and the 38th day of the experiment, before any group showed a decline in food intake. Such a decline started on the 38th day for group C, on the 40th day for other deficient groups.

These results do not support the hypothesis that vit. A intervenes directly in the synthesis of cystine, as claimed by Meunier and his associates. For one thing, while the inclusion of cystine in the diet did increase the maximum weight gain of the animals fed vit. A deficient diets, it did not delay the onset of the deficiency. As a matter of fact, weight loss occurred earlier in group C and symptoms were more acute among the animals of this group. Secondly, adding bromobenzene to the vit. A-deficient diets did not cause a more drastic effect than in non-vit. A-deficient animals. If vit. A was specifically instrumental in the synthesis of cystine, one would expect the vit. A-deficient animals, whose capacity of cystine synthesis should already be impaired, to be more immediately and more severely affected by the bromobenzene.

Summary. A study of the effect of bromobenzene administration to vit. A-deficient animals on low-protein diets, supplemented or not supplemented with cystine, and to their controls, does not support the theory, postulated by some authors, that vit. A is directly implicated in the synthesis of cystine.

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Manometric and Histochemical Demonstration of Tyrosinase in Foetal Guinea-Pig Skin. (19495)

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At least some of the skin and hair pigments of mammals (melanins) appear to be produced from the precursor, tyrosine, and it has recently become possible to demonstrate tyrosinase activity in normal mammalian skin. A histochemical demonstration of melanin formation from tyrosine was made in the case of non-pigmented human skin subjected to erythema doses of ultra-violet irradiation(1), and tyrosinase activity has also been demonstrated in skin homogenates from mice and foetal guinea pigs by means of manometric measurements of oxygen consumption(2-4). Since this pigment-forming process has been demonstrated either histochemically or manometrically, it was considered desirable to attempt to demonstrate both aspects of the process in the same sample of tissue.

Methods. In the experiment reported here the intense brown portion from the back of an approximately 52-day-old brown-and-white-spotted foetal guinea pig was separated from the white portion, placed in a frozen mortar surrounded by dry ice, ground to a frozen powder by means of a previously frozen pestle, and this frozen powder was subsequently suspended in pyrex-redistilled water. Four Warburg reaction vessels, each with a volume of approximately 10 ml, were used. Each vessel contained 0.2 ml of 20% KOH in the center

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well, as well as 1 ml of skin suspension in the main portion of the vessel. Each sidearm of two of the vessels contained merely 0.5 ml of M/10 phosphate buffer, pH 6.8 (control vessels), while each sidearm of the other vessels contained 0.5 ml of L-tyrosine dissolved in the same buffer, at a concentration of 0.5 mg/ml (experimental vessels). After 10 minutes of temperature equilibration in a water bath at 38° C, the sidearm contents were tipped into the main portions of the vessels (zero time) and oxygen consumption measurements were obtained. The *net oxygen consumption,* attributable to the enzymatic oxidation of tyrosine, was obtained by subtracting the mean endogenous oxygen consumption of the controls from the mean oxygen consumption in the experimental vessels. Immediately after the Warburg run, skin fragments from one control vessel and from one experimental vessel were removed and fixed in 1:10 formol-alcohol (approximately 15 minutes), dehydrated in absolute ethyl alcohol (approximately ½ hour), cleared in benzene (overnight) and mounted in Permount (Fisher Scientific Co.). Photomicrographs of the experimental and control skin fragments were made under low-power magnification under as equivalent conditions of plate exposure as possible; the two sets of fragments being mounted on the same slide, thus necessitating only a change of fields for the 2 different photographs.

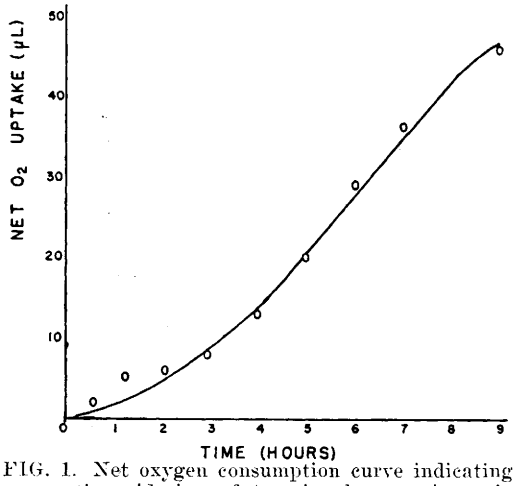

FIG. 1. Net oxygen consumption curve indicating enzymatic oxidation of tyrosine by tyrosinase in brown skin (see text).

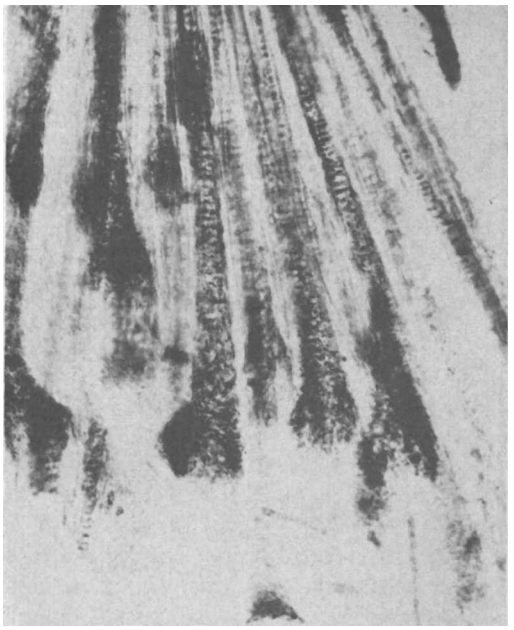

FIG. 2. Whole mount of brown skin fragment in the absence of tyrosine (controls). Note the relatively uniform pigmentary details extending from the hairs down to the hair bulbs.

Results. The curve for the net oxygen consumption, indicating the enzymatic oxidation of tyrosine, is shown in Fig. 1. (Other tests (4) have demonstrated specificity of the skin catalyst for the L-configuration of tyrosine, poisoning by phenylthiourea, and enhancement of net oxygen consumption and pigment-production by iodoacetamide.) Correlated with this oxygen consumption was the formation of a brown-black pigment suspension in the experimental vessels, as well as a marked darkening of the larger skin fragments in these vessels. Histological comparison of skin fragments from experimental and control vessels revealed a marked difference in the intensity of hair bulb pigmentation. The control hair bulbs contained brown pigment, but were not completely filled with this pigment. The experimental hair bulbs, on the other hand, appeared to be almost choked with very dark brown pigment. Moreover, this intense pigmentation extended for some length distal to the hair bulb. These differences are indicated in the photographs shown in Fig. 2 (control) and 3 (experimental).

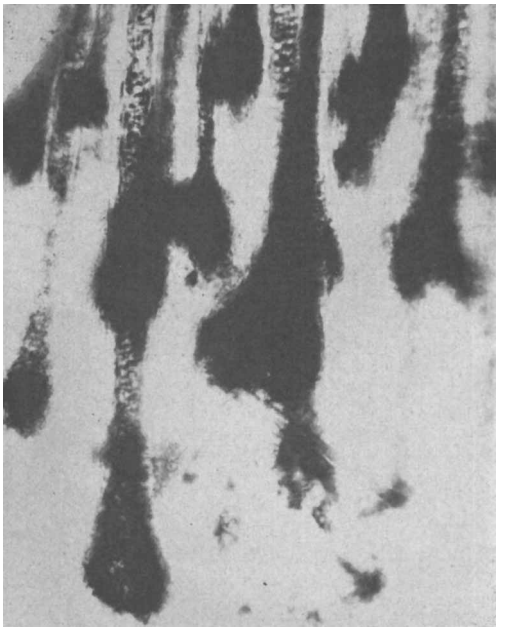

FIG. 3. Whole mount of brown skin fragment in the presence of tyrosine (experimentals). Note the very densely pigmented hair bulbs and lower hair portions. Note also upper hair portions, showing the same pigmentary details as found in the control hairs.

Thus, presumably for the first time, are demonstrated both the enzymatic oxidation of, and pigment-formation from, tyrosine, in the same sample of normal mammalian skin.

Discussion. The positive results reported here strongly indicate that at least some of the dark mammalian hair and skin pigments are products of the tyrosinase system. Studies on a rather extensive series of guinea-pig coat-color genotypes fail, however, to reveal any simple parallelism between tyrosinase or dopa oxidase activity and genetically determined amount or kind of natural pigment(4). These studies, which will be reported and considered in detail elsewhere, confirm in certain major respects previous studies of dopa reactions in the case of guinea-pig skin(5,6).

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Cultivation of Lansing Poliomyelitis Virus in Tissue Culture. II. Utilization of Glucose in Synthetic Medium.* (19496)

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(Introduced by R. C. Parker.)

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The work of Enders and his associates (1-3) has indicated that poliomyelitis virus can be propagated in cultures of human embryonic tissues. In their work, Hanks-Simms' fluid was used as the source of nutrient. Recently, we have shown(4) that this fluid can be replaced by a synthetic nutrient medium, mixture No. 199, devised by Morgan, Morton, and Parker(5,6). Mixture 199 is of chemically defined composition, and allows of survival of suspended tissue fragments and proliferation of virus for a prolonged period. Hitherto, metabolism has been followed by observing changes in pH. Enders, for example, has reported that the pH values of uninfected cultures are lower than those of infected cultures. Since the Meyerhof sequence of glycolysis has been found to apply to almost all biologic systems, it seemed appropriate to

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