proteins, as shown by the benzidine reagent (Fig. 4), corresponded to the location of the plasma radioactivity (Fig. 5), indicating that the radioactivity had moved with the plasma proteins. When the radioactivity extracted by butanol was chromatographed with butanol developers, it proved to consist almost exclusively of radiothyroxine. However, when chromatographed with buffers, this radiothyroxine remained at the origin (Fig. 6). Nevertheless, if the radiothyroxine was added to nonradioactive plasma, it was displaced (Fig. 8) along with the plasma proteins (Fig. 7), in a manner similar to that of the original radioactive plasma (Figs. 4 and 5).

The same results were obtained when nonradioactive thyroxine was chromatographed with plasma that did not contain hemin, indicating that presence of hemin was not necessary for plasma-thyroxine combination.

Interpretation. These results demonstrate that, besides thyroglobulin and iodide, the thyroid gland contains small amounts of monoiodotyrosine, diiodotyrosine, and thyroxine (Fig. 1). It has been shown that the presence of these amino acids is not due to exchange phenomena (Fig. 3) or to radiochemical action. The presence in the thyroid of these amino acids, which are known to be components of thyroglobulin, lends support to the hypothesis that thyroglobulin undergoes proteolysis by the enzyme described by De Robertis (*1*).

The thyroxine found in plasma is most likely of thyroid origin. This is indicated by experiments showing that the butanol-soluble radioactivity of plasma is extremely low in thyroidectomized rats (*3*). The passage of thyroxine into the circulation has been previously explained by a gradient of thyroxine concentration in thyroid tissue and plasma (*4*). It may be pointed out further that the diffusion of thyroxine from the thyroid would tend to correct the increase in osmotic tension due to proteolysis in the colloid. The failure to find significant amounts of the other iodinated amino acids in plasma may be explained by their destruction within the follicle and a reutilization of their iodine by the gland.

In the plasma, thyroxine has been found combined with proteins. This combination may be severed easily by butanol, but is reconstituted when thyroxine is placed in contact with plasma proteins (Figs. 7 and 8). Furthermore, since plasma proteins may combine with amounts of thyroxine well above the physiological doses (100 μg in 0.01 ml), it may be assumed that under physiological conditions the thyroxine secreted from the thyroid combines with plasma proteins as it is released.

The nature of the thyroxine complex in plasma is obscure. However, iodine has been found in the albumin fraction of plasma (*6, 8, 9*), and to a somewhat lesser extent in the globulin fraction (*6, 8*). Attempts to determine the plasma constituent which is the thyroxine carrier are currently under way.

In summary, when adult female rats on a low iodine intake were sacrificed 48 hr after an injection of carrier-free radioiodide, butanol extracts of unhydrolyzed thyroid and plasma analyzed by radioautography of two-dimensional paper chromatograms revealed the presence of six radioactive compounds besides iodide and thyroglobulin. Of these, three were identified as thyroxine, diiodotyrosine, and monoiodotyrosine. In the blood plasma, practically all the butanol-extractable radioactivity was present as thyroxine, with a very low amount of diiodotyrosine and iodide. When the whole plasma was analyzed by radioautography of two-dimensional buffer chromatograms, it was shown that the location of the radioactive material corresponded to that of the plasma proteins. Although thyroxine solutions showed no movement from the origin under these conditions, radioactive thyroxine dissolved in nonradioactive plasma was displaced along with the plasma proteins and gave a pattern very similar to that of radioactive plasma itself.

It was concluded that thyroxine, after its release by the thyroid gland, circulates in combination with plasma proteins. The complex thus formed can be split with butanol and reconstituted *in vitro*.

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Xanthopterin Obtained from the Skins of the Yellow Mutant of *Bombyx mori* (Silkworm)

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The gene named "Lemon colored" which is present in the third chromosome of *Bombyx mori* gives rise to yellow-colored larvae instead of the white ones of the normal type. The yellow pigment exists in the epidermis and is considered to possess a close relationship with tryptophan metabolism, as in the case of the eye pigments of *Drosophila* and other insects (*4, 5*). Chemical researches made on this pigment have identified it as xanthopterin.

A hundred larvae of the yellow *lem* type in the fifth instar were dissected, the skins were denatured by treatment with alcohol, the lipochromes were extracted with ether in a Soxhlet apparatus, and the pigments finally were extracted with 200 ml and 400 ml of boiling water. The first extract gave a purple-plus-yellowish green fluorescence, and the second a purple fluorescence. The first extract was made acidic (0.05*N*) with hydrochloric acid and left overnight in an icebox, when 160 mg of white crystals was obtained. These crystals, after recrystalliza-

[1] The chemical researches of this report were carried out by Hirata and Nakanishi.

[2] The authors wish to acknowledge the helpful suggestions of Prof. F. Egami during the course of this research.

tion (accomplished by pouring water into the concentrated sulfuric acid solution), were identified as uric acid through their ultraviolet absorption spectrum[3] (absorption maximum: 280 mμ in 0.1% sodium hydroxide) and certain qualitative properties. The filtrate, together with the second 400-ml extract, was evaporated *in vacuo*, and the residue was washed with 2*N* hydrochloric acid. The properties of the insoluble portion (white crystals and powder) will be described.

The hydrochloric acid solution was evaporated *in vacuo*, water was added, and the solution was then made alkaline with ammonia, the insoluble portions being discarded each time. After final refinement by the addition of charcoal, the green fluorescent solution was submitted to paper chromatography, using the butyl alcohol-acetic acid mixture by Partridge (*6*). The bands were located by their fluorescence (*2*). Since pure xanthopterin was unavailable, paper chromatography of the wings of a pierid (*Eurema etaeta* Boisduval) known to contain xanthopterin was carried out simultaneously. Though Good and Johnson (*2*) have given 0.38 for the R_f value of xanthopterin, we have obtained a value of 0.54. The yellow pigment from *Bombyx mori* gave the same R_f value, and several qualitative properties showed it to be xanthopterin. Riboflavin also gave the same R_f value, but its fluorescence and chemical behavior were different.

It is to be noted that, when left in the sunlight for one day, both the silkworm and pierid extracts acquired a fluorescence of a bright blue tone and instead of the yellowish green band on the paper chromatogram a bright blue band with an R_f value of 0.26 appeared. Similarly, when the paper-chromatographed filter strip of the undecomposed solution was left in the sunlight, the yellowish green band changed into a band with a bright blue fluorescence. The reason for this kind of photolysis of xanthopterin is obscure and apparently has not been described in the literature.

Xanthopterin was also concentrated from the original boiling water extract, according to the method of Crammer (*1*), using liquid phenol. This solution gave a band on the paper chromatogram with a purple fluorescence (R_f: 0.50) corresponding to the pterinlike substance mentioned below, in addition to the band of xanthopterin. One hundred milliliters of water was added to the 2*N* hydrochloric acid-insoluble portion mentioned above, the rather heavy crystals (uric acid) were separated, and the remaining flocky precipitate was reprecipitated several times from boiling water. The filtrate was next dissolved in a small amount of concentrated sulfuric acid and was poured into water dropwise, when white crystals were obtained. The hot aqueous solution gave a strong purple fluorescence (R_f: 0.50).

Thus, xanthopterin, uric acid, and a minute amount of some white crystals have been obtained from the yellow mutant. This white crystal, though closely related to leucopterin, isoxanthopterin, and the purine homologue, is not identical with any of them, and seems probably to be a new pterin. The chemical researches on this substance are being carried out and will be reported subsequently. This substance is obtained in greater amount from skins of the normal type of silkworms.

Jucci (*3*) has stated that the white epidermis pigment is a urate, and Shimizu (*7*), basing his work on this view of Jucci, has made some quantitative studies of the uric acid production by means of the Folins-Denis-Wu reaction. However, since the new pterinlike substance also gives a strongly positive Folins-Denis-Wu reaction, the results of Shimizu should be given further consideration.

We are not yet in a position to discuss the formation of epidermis pigments from a genetic point of view until the chemical structure of this substance is clarified.

[3] The authors are greatly indebted to Prof. K. Yamasaki for the measurements.

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Processing Unit for 35-mm Color Film[1]

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The advantages of 35-mm color film are of such importance that its use is steadily increasing as a means of scientific record. Color recording has its greatest value where a combination of colors and shapes is such that in each separate item changes may exist which are hardly measurable and yet which, taken together, give a very marked visual difference in the total picture. This is of course especially true of pathological changes. At the present time, however, few facilities have been created for the average worker to develop his own films. This may be necessary in cases where the original picture will disappear before commercial development is completed and thus be lost. Having to face such a problem ourselves, and finding no available answer, we were compelled to find its practical solution. As this has been rather satisfactorily accomplished, it occurred to us that others might wish to take advantage of our study.

Very briefly, we may remark that to process color film requires 13 separate operations, one being a reexposure, four being intermediary or final washing. For the commercial operator with adequate volume of work, large and expensive tanks are available, but these are of little value to the ordinary laboratory, as the cost in chemical replacement alone would be prohibitive. For the amateur there are small tanks fitted with spools on which film must be placed. After a few trials with this aggravating and inefficient apparatus, we hastily discarded it. What was needed was an intermediate arrangement that could handle one or two films per day, be economical in operation, fully and evenly expose the film to the processing

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