The experiments, which began at the Chemical Department, Cancer Research Laboratories, Hebrew University, Jerusalem, are at present being followed up at the Department of Biological and Colloidal Chemistry of the same university, by the kind permission of Prof. A. Fodor, director of the Department.

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[1] Feigenbaum, J., "Food Industries, Chemistry and Processing" (New York, 1946), in the press.
[2] Holmes, H. N., Corbet, R. E., and Hartzler, E. R., *Ind. Eng. Chem.*, **28**, 133 (1936).
[3] Olcott, H. S., and Mattill, H. A., *J. Amer. Chem. Soc.*, **58**, 1627, 2204 (1936).
[4] Hilditch, T. P., *Chem. and Ind.*, **67** (1944).
[5] Lea, C. H., *J. Soc. Chem. Ind.*, **63**, 107 (1944).
[6] Lovern, J. A., *J. Soc. Chem. Ind.*, **63**, 13 (1944).

Constitution of Hair Melanins

MELANINS can be extracted from hair by dilute caustic soda solution and purified by chromatography on calcium and magnesium carbonates[1,2]. The extraction with 0·05 *N* caustic soda is easier with rabbit hair and sheep wool than with human head hair. Owing to this ease of extraction, which is important in order to obtain pure melanins, but also because we had in mind an analysis of the phenogenesis of melanic pigmentation[3,4], we have studied chiefly melanins from rabbits of known genotypic constitution. Nevertheless, the similarity of the data for mammalian hair melanins generally with those for rabbit hair melanins suggests that our conclusions are applicable to hair melanins in general and to melanins of other tissues.

The purest melanins have been prepared in the following way: rabbit hairs are well cleaned and treated with alcohol and ether, and extracted with 0·05 *N* caustic soda for a week or less at 37° C., in glass-stoppered flasks (in general, 3 gm. of hair to one litre of solvent). The extraction is stopped before the hairs disintegrate into separated fibres; the purest melanins are obtained in the first three or four days of extraction. The solution is afterwards filtered and chromatographed on calcium carbonate (Merck *praec. leve*) if the rabbit is of the black group (black, wild and grey); or on magnesium carbonate (Pattinson's carb. of magnesia, England) when dealing with brown rabbits or rabbits of the yellow group (yellow, orange and sand). Brown and yellow melanins are not efficiently adsorbed on calcium carbonate, whereas magnesium carbonate retains all kinds of pigment. Development of the chromatograph is by *N* caustic soda, the melanin being tenaciously adsorbed in the upper layer, while disintegrated keratins and more or less decomposed pigment pass through the column[1,3]. Instead of elution, which is not possible after adsorption in these conditions, the melanin is isolated by treating the carbonates with dilute hydrochloric acid. The melanin is now dissolved in 0·05 *N* caustic soda and precipitated with dilute hydrochloric acid; solution and precipitation are repeated a further three times. The final product is well washed with distilled water.

The pigments extracted and purified in this way are easily soluble at *p*H 7·5 or more, for example, in a chloride–bicarbonate buffer. They are melanoproteins formed by closely bound proteins and melanoids[1,3], these latter being the coloured part of the melanins. We have not yet succeeded in decomposing the melanoprotein into its protein and melanoid components, but we were able, by hydrolysis of the protein, to obtain the melanoid intact. After hydrolysis for 48 hr., the melanoid is dried and weighed. For the rabbit the results in Table 1 were obtained.

TABLE 1.

Melanin	Black	Brown	Yellow
Melanoid	55·5 ± 1·0%	41·6 ± 2·0%	30·3 ± 1·7%
Protein (by difference)	44·5	58·4	69·7

Nitrogen determinations (by Kieldahl) agree with the results of the hydrolyses. The following is an example for black melanin: 96·74 mgm. of melanin with 10·4 per cent nitrogen, or a total of 10·061 mgm. nitrogen, was hydrolysed with 20 per cent hydrochloric acid for 48 hr.; after hydrolysis, 56·62 mgm. of melanoid was obtained with 7·0 per cent, or 3·963 mgm. nitrogen; in the hydrolysate we found 6·275 mgm. nitrogen, while the nitrogen calculated for protein of 15·8 per cent nitrogen is 6·339 mgm.

The melanoids which remain after hydrolysis are almost insoluble in 0·05 *N* caustic soda, but they dissolve when treated with *N* caustic soda for some hours. After dissolution they are precipitated with dilute hydrochloric acid and the dissolution and precipitation are repeated twice. The purified melanoids dissolve readily in 0·05 *N* caustic soda and are different for the three kinds of melanins.

The absorption of the original melanins (melanoproteins) compared with that of the purified melanoids gives results which are in relatively good accord with those of the hydrolyses. According to the colorimetry results at 515 mμ, black melanin has about 60 per cent melanoid and 40 per cent protein, whereas yellow melanin is composed of 30 per cent melanoid and 70 per cent protein. The difference between these values and those of the hydrolyses may be due to a little humin which is adsorbed by the melanoid.

Analyses of the amino-acids of the protein part of the melanoproteins have given the results summarized in Table 2, in which column *a* shows the weight of the amino-acid as a percentage of the dry weight of the protein and column *b* gives the amino-acid nitrogen as a percentage of the protein nitrogen determined in the hydrolysate, assuming that the proteins contain 15·8 per cent nitrogen. The analyses of brown melanin are not yet completed.

We have also analysed white rabbit hair keratin and merino wool by the same methods, and found results in accord with those of the literature for wool keratins.

Comparing the amino-acid composition of the proteins of the melanoproteins with that of the keratins, it is obvious that the melanoproteins

TABLE 2.

Rabbit melanin	Black		Yellow	
	(*a*)	(*b*)	(*a*)	(*b*)
Arginine	6·67	13·59	10·81	22·03
Histidine	1·01	1·73	0·97	1·67
Tyrosine	2·44	1·20	5·52	2·70
Tryptophane	2·68	2·33	3·97	3·45
Cystine–Cysteine	1·21	0·89	0·80	0·59
Methionine	3·24	1·93	1·18	0·70

are not melanokeratids as has been claimed by Stary and Richter[5,6]. By their composition and solubility the melanoproteins are similar to non-fibrous proteins and may be perhaps regarded as belonging to the pseudo-globulins. Like the rabbit melanins, human hair and sheep wool melanins (and also probably *Sepia* melanin) are proteins with a protein and a melanoid part. It seems to us that we can safely conclude that the natural hair melanins are dual compounds of protein and melanoid; the melanoids are perhaps similar to 'synthetic melanins' prepared by several authors[7,8] from tyrosine, phenylalanine and tryptophane. The results of Greenstein, Turner and Jenrette[9] can perhaps be better explained by assuming that natural melanins are proteins formed by protein and melanoid, rather than by supposing a 'contamination' of a pseudo-globulin with a 'melanin'.

Another conclusion from this work is the diversity of the melanins from hairs of different colorations; the difference shown between the keratins and the protein parts of the pigments is also important in connexion with an understanding of certain steps of the phenogenesis of melanic pigmentation.

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[1] Serra, J. A., *Rev. Fac. Cienc. Univ. Coimbra*, **7**, 235 (1939).
[2] Serra, J. A., *Las Ciencias, Madrid*, **6**, 904 (1941).
[3] Serra, J. A., *Genetica*, **23**, 300 (1943).
[4] Danneel, R., *Ergeb. Biol.*, **18**, 55 (1941).
[5] Stary, Z., and Richter, R., *Z. Physiol. Chem.*, **253**, 159 (1938).
[6] Richter, R., and Stary, Z., *Arch. Dermat. Syphil., Berlin*, **178**, 373 (1939).
[7] Evans, W. C., and Raper, H. S., *Biochem. J.*, **31**, 2155 and 2162 (1937).
[8] Spiegel-Adolph, M., *Biochem. J.*, **31**, 1303 (1937).
[9] Greenstein, J. P., Turner, F. C., and Jenrette, W. V., *J. Nat. Cancer Inst.*, **1**, 377 (1941).

Action of Thyroxin and the Response of the Thyroid to Treatment with Sulpha Drugs

THIOUREA and derivatives[1–3] and sulphonamide compounds[4,5] inhibit the formation of the catabolic hormone both in normal and in hyperactive thyroids. In a vain attempt to compensate for the lack of hormone, the gland undergoes hyperplasia, and hypothyroidism with hyperplastic goitre can result[1,6]. A few isolated results suggest that the possibility of the drugs also antagonizing the circulating hormone cannot be excluded[7].

Concerning this question, two series of experiments were carried out. The first was designed to investigate whether the protection afforded by thyroxin against procaine[8,9] is influenced by sulpha drugs. Three groups of young female albino mice of 20 gm. body weight, so far as possible litter mates, received respectively:

(1) A sulpha drug for 11 days at the rate of 5 mgm. per mouse per day. Thiouracil, 4-methyl-2-thiouracil—both known to exert anti-thyroid activity[10]—and two other sulpha-pyrimidine compounds, referred to as *B*2 and *B*8, were given, one of which (*B*2) showed bacteriostatic properties. From the seventh day of treatment, a 0·1 per cent solution of sodium thyroxin in a daily dose of 0·1 mgm. per 20 gm. body weight was added. Each dose of thiouracil and methylthiouracil was suspended in 0·1 ml. of a solution of gum acacia and injected intraperitoneally; *B*2 and *B*8 were dissolved in 0·1 ml. of propylene glycol and, like the thyroxin, injected subcutaneously.

(2) Thyroxin as group (1), but without any sulpha drug.

(3) No treatment.

Twenty-four hours after the last injections, the colonic temperatures of all the mice were recorded three times at 15-min. intervals. Afterwards a 2 per cent solution of procaine hydrochloride was injected subcutaneously in a dose of 3 mgm. per 20 gm. body-weight. After this injection, recording of body temperature was continued for two hours.

MAXIMAL FALL OF COLONIC TEMPERATURE DURING TWO HOURS AFTER SUBCUTANEOUS INJECTION OF 3 MGM. OF PROCAINE HYDROCHLORIDE PER 20 GM. BODY-WEIGHT

No. of mice	Mean fall of temperature ° C.	Variation ° C.	Error of the mean	Treatment
20	3·8	2·3–6·2	1·00	none
31	1·5	0·0–2·7	0·66	thyroxin
16	2·6	1·4–3·4	0·69	thyroxin + thiouracil
15	2·8	0·8–4·8	1·03	thyroxin + 4-methyl-2-thiouracil
20	2·7	1·0–4·8	0·89	thyroxin + *B*2
15	2·2	1·2–3·7	0·74	thyroxin + *B*8

The protection exerted by thyroxin (see table) appeared to be smaller in the mice prepared with the sulpha drugs than in those which received thyroxin alone. But only the difference in response between the latter and those treated with thiouracil was statistically significant. It may be of interest that thyroxin significantly antagonizes