

the injection. Following freeze-drying and fixation in Carnoy's solution the tissues were embedded in paraffin, sectioned<sup>5</sup>, and stained with haematoxylin-eosin.

The following are the results of our histological examinations (see Fig. 1): (1) In both the trauma and MSH groups there was, without exception, a swelling of the anterior ciliary processes, an interstitial oedema and possibly a dilatation of the small intraciliary vessels. Typical 'Greeffsche Blasen' with splitting of the two layers of the secretory epithelium<sup>6</sup> were observed to a similar extent in both groups. In the 17 normal eyes no such changes were seen.

(2) In 7 eyes from MSH-treated animals, where no flare effect occurred, there was still a marked swelling of the ciliary processes, but no 'Greeffsche Blasen' were found.

(3) The trauma effect was observed around the whole circumference of the ciliary body but more excessively on the upper part, that is, behind that part of the iris treated with infra-red irradiation.

(4) No changes in the iris, not even in its irradiated part, or in the melanocytes of the iris and ciliary body could be observed in any of the eyes.

Although recent investigations<sup>7</sup> have shown that MSH causes a series of general effects in the rabbit, for example, a decrease in the blood pressure and dilatation of the peripheral vessels, there is still no evidence that the permeability disturbance should be a generalized effect.

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<sup>2</sup> Dyster-Aas, H. K., and Krakau, C. E. T., *Endocrinology*, **74**, 255 (1964).

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### Permeability Effects of $\alpha$ -Melanocyte-stimulating Hormone

$\alpha$ -MELANOCYTE-stimulating hormone ( $\alpha$ -MSH) induces an increased permeability of the blood-aqueous barrier of the rabbit eye<sup>1,2</sup>. It has been shown in the preceding communication that  $\alpha$ -MSH produces a swelling of the ciliary processes with an interstitial oedema in this species. Since the permeability disturbance might be part of a more generalized effect, it was considered worthwhile investigating whether or not there was also an increased leakage of proteins through other barriers.

<sup>125</sup>I-labelled human serum albumin (Radiochemical Centre, Amersham) was given intravenously 30 min after a subcutaneous injection of 10  $\mu$ g/kg synthetic  $\alpha$ -MSH (ref. 3) (supplied by Ciba, Basle) in 0.9 per cent sodium chloride to 12 pigmented rabbits and after the same volume of 0.9 per cent sodium chloride had been given to 5 pigmented rabbits (controls). Blood samples were collected at 30-min intervals. The aqueous flare response<sup>3</sup> was taken as proof of the local permeability disturbance of the eye after the  $\alpha$ -MSH and was estimated roughly by means of focal illumination before and 2 h after the injection. 2.5 h after the  $\alpha$ -MSH injection the rabbits were killed, their eyes enucleated and dissected essentially as described previously<sup>4</sup>. Small tissue samples were also collected from various organs. The radioactivity of the blood and tissue samples was determined.

The radioactivity in counts per minute of the tissue samples was divided by the radioactivity in c.p.m./ $\mu$ l. plasma in the blood sample obtained immediately before the animals were killed. This gave a figure for the apparent

Table 1. THE RELATIVE FALL IN BLOOD RADIOACTIVITY AFTER AN INJECTION OF <sup>125</sup>I-ALBUMIN

In animals showing an aqueous flare response after the injection of  $\alpha$ -MSH, R; in animals not responding with aqueous flare, NR; and in controls, C. Arithmetic means  $\pm$  the standard error of the means. The radioactivity of the first blood sample is 1.00

Time after <sup>125</sup> I- alb. inj.	4 min	30 min	60 min	90 min	120 min
R	1.00	0.90 $\pm$ 0.0086	0.83 $\pm$ 0.0114	0.79 $\pm$ 0.0125	0.77 $\pm$ 0.0191
NR	1.00	0.87 $\pm$ 0.0114	0.79 $\pm$ 0.0187	0.75 $\pm$ 0.0196	0.71 $\pm$ 0.0200
C	1.00	0.89 $\pm$ 0.0205	0.82 $\pm$ 0.0249	0.77 $\pm$ 0.0232	0.74 $\pm$ 0.0190

Table 2. THE APPARENT PLASMA CONTENT OF THE ANTERIOR UVEA AND THE CHOROID PREPARATIONS AND IN 1 ML. AQUEOUS HUMOUR

In animals responding with increased aqueous flare to  $\alpha$ -MSH, R; in non-responsive animals, NR; and controls, C. Arithmetic mean  $\pm$  the standard error of the mean

	The apparent plasma volume ( $\mu$ l.)		
	Anterior uvea	Choroid	1 ml. aqueous humour
R	33.5 $\pm$ 2.83	6.03 $\pm$ 0.66	265.2 $\pm$ 33.9
NR	7.1 $\pm$ 0.64	4.40 $\pm$ 0.32	3.4 $\pm$ 0.70
C	7.1 $\pm$ 1.27	4.81 $\pm$ 0.26	6.6 $\pm$ 2.90

plasma content, which included 'plasma' both from intravascular and extravascular compartments.

In 6 of the 12 rabbits which had been given MSH there was a pronounced increase in aqueous flare. In the remaining 6 and in the control group no aqueous flare was detected.

We found no increased rate of loss of <sup>125</sup>I-albumin from the intravascular compartment (Table 1). In the 6 rabbits where the aqueous flare was prominent, the albumin content of the aqueous humour was very much increased as compared with the 6 test rabbits in which no flare was observed and with the controls (Table 2). The apparent plasma content of the anterior uvea was greatly increased, while no such effect was observed in the choroid as shown in Table 2. No similar change in apparent plasma content could be found in the urine, the gall, the brain, the liver, the kidneys, the lung, the spleen, the intestines, the suprarenals or the muscles.

It thus seems that the permeability increasing effect of the melanocyte-stimulating peptides is rather specific for the eye. However, it should be pointed out that a rise in the vasomotor tone of the small veins might be of importance in the increased protein transfer.

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### The Ussing Relationship and Chemical Reactions: Possible Application to Enzymatic Investigations

It may not be widely appreciated that the Ussing flux ratio relationship<sup>1,2</sup> is applicable to chemical reactions in general. It is possible that measurement of such flux ratios may yield information about reaction intermediates, particularly in enzymatic processes, but perhaps also in catalytic, heterogeneous, and other chemical reactions.

To demonstrate the relationship of the Ussing criterion to a typical chemical reaction it is convenient to use an argument based on induced transport<sup>3</sup>. Consider the reversible reaction:

