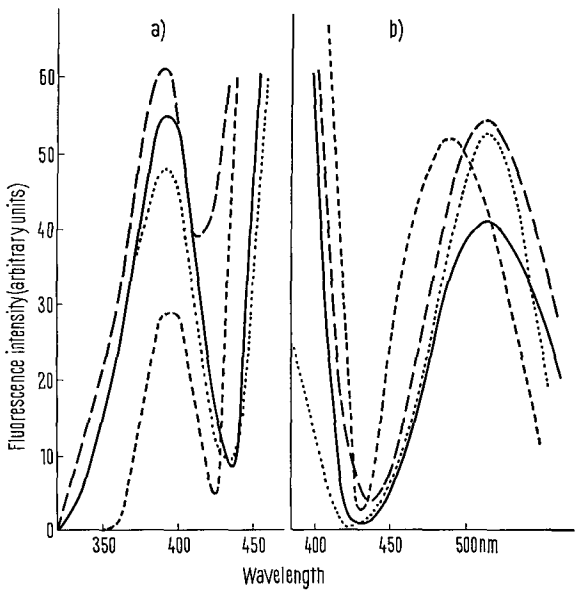
Table II. Chromatographic characteristics of the spots

Substances	UV-visualization 254 nm	366 nm	NNCD[a]	*p*-dimethylaminobenzaldehyde[b] 30 min after spray	24 h after spray	Rf
Fasciola hepatica extract	opaque immediately and fluorescent 24 h later	not fluorescent	orange/yellow	purple[c]	blue-green	0.58
5-HT[d]	opaque	not fluorescent	peach-red	blue/violet	blue-grey	0.70
6-HT	opaque	fluorescent	ruby-red	blue	blue-green	0.72
5-HTP	opaque	not fluorescent	flesh-colour	yellow	yellow/grey	0.50
4-HTP	opaque	not fluorescent	red-brown	grey/yellow	grey	0.55
5-MTP	opaque	not fluorescent	gold/yellow	grey	grey-blue	0.58
5-acO-N-acTP	opaque	not fluorescent	yellow/orange	purple	rose	0.74
5-meOT	opaque	not fluorescent	gold/yellow	violet	blue-green	0.72
T	opaque	not fluorescent	yellow	purple	blue-green	0.82

[a] 0.1 g of 2-chloro-4-nitrobenzenediazonium naphthalene-2-sulphonate were dissolved in 1 ml of concentrated HCl and the volume was brought to 100 ml with distilled H_2O. [b] 20 ml of *p*-dimethylaminobenzaldehyde 5% ethanolic solution were added to 10 ml of concentrated HCl and the volume was brought to 100 ml with absolute ethanol. [c] During the spray development the spot appears yellow and then becomes purple. [d] 5-hydroxytryptamine creatinine-sulphate.



Standard = 5-hydroxytryptamine creatinine-sulphate. (———), Fluorescence spectra of extracted standard; (- - - - - -), *F. hepatica* tissue extract; (– – – –), extracted standard added with *F. hepatica* extract; (.....), unextracted standard. (a) Activation spectrum. (b) Fluorescence spectrum.

fluorometric and biological tests. According to our present results, on the contrary, the quantitative data of MANSOUR[1] cannot be confirmed. From our experiments it can moreover be seen that the samples of incubated liver flukes show about 48% reduction ($P < 0.001$) in their content of the fluorescent compound, when compared with the samples of freshly collected worms. In our opinion this fact is worth noting, although, at present, we cannot explain this rapidly occurring decay.

Whether the compound described in this paper is itself the true neuromuscolar transmitter in *F. hepatica* or a precursor of the true transmitter we cannot yet say. However, the occurrence in *F. hepatica* tissue of a compound strictly related to the group of the indole compounds provides further evidence that these worms might account for it for their biological needs, and once more supports the suggestions of previous works[1-4] about the possible role of 5-HT or a related substance for the transmission of nerve impulses in *F. hepatica*[10].

Riassunto. Gli AA. descrivono le caratteristiche fluorimetriche e cromatografiche di un estratto butanolico dal tessuto di *F. hepatica* concludendo che la sostanza estraibile presenta caratteristiche indoliche ma non appare identificabile nè con la 5-HT nè con alcuni altri indoli saggiati.

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[10] Ricerche svolte con il contributo del C.N.R.

Intermedin (MSH)-Like Effect of a Thermal Polymer on Vertebrate Chromatophores

The ability of small intermedin (MSH) peptides to stimulate dispersion of pigment granules within frog melanophores has been reported[1,2]. Iridophore contraction (reflecting platelet aggregation) is also induced by these peptides and this implies that their action is essentially like that of the parent hormone and suggests that mechanisms of both melanophore and iridophore stimulation have common features[3]. Recently, FOX and WANG[4] reported that thermal polymers of arginine, glutamic acid, glycine, histidine, phenylalanine, and tryptophan have melanophore-stimulating activity. The present study was undertaken to determine whether such thermal

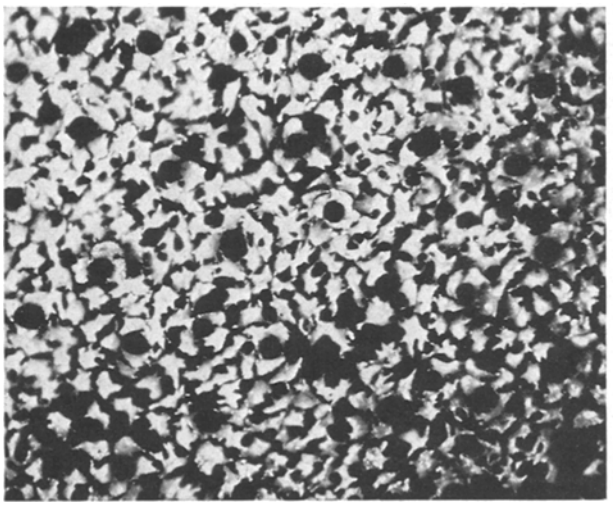

Fig. 1. Photomicrograph (by reflected light) of iridophores of *Rana pipiens* skin maintained in Ringer solution. Reflecting platelets are dispersed intracellularly resulting in a flat and extensive reflecting surface. Punctate melanophores beneath the iridophores are barely visible. × 360.

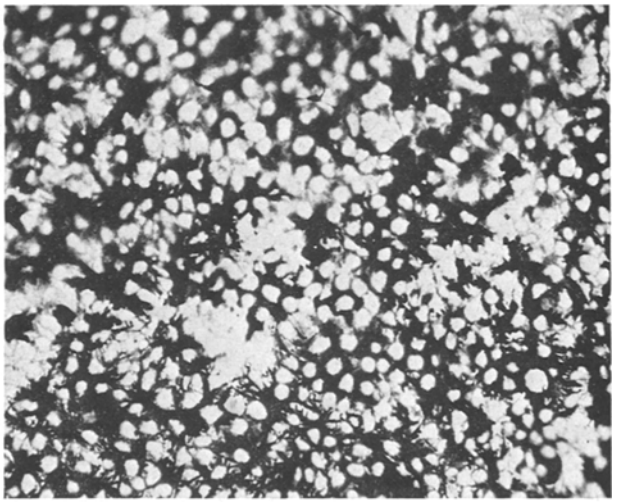

Fig. 2. Photomicrograph (by reflected light) showing response of iridophores of *Rana pipiens* skin to a thermal polymer of amino acids possessing intermedin-like activity. An intracellular aggregation of reflecting platelets within iridophores results in a rounded appearance with reduced reflecting surface. Dendritic melanophore processes can be seen overlapping iridophores. × 360.

polymers might similarly stimulate iridophores and whether or not such effects are normal rather than pathological and are truly intermedin-like.

Methods and materials. Leg and thigh skins were removed from the frog, *Rana pipiens*, and back skins of the lizard, *Anolis carolinensis*, and were tested for their in vitro response to a thermal polymer of: arginine, glutamic acid, glycine, histidine, phenylalanine, tryptophan. The change in color of these skins in response to the agents used in this study was detected by a photometric reflectance method as originally described for the in vitro bioassay of MSH[5-7]. The initial average reflectance value for each group of skins was given a value of 100% and all succeeding average values for each group of skins were recorded as percentage changes above or below this initial base reading[9]. 3 separate experiments were performed and each experimental group within any experiment consisted of at least 6 skins from as many individual frogs or lizards. Skins were prepared as whole mounts for microscopical observation as previously described[6].

Results. Concentrations of 10^{-5} and 10^{-6} g/ml of the thermal polymer failed to induce iridophore contraction in frog skins; however, concentrations of 10^{-4} and 5×10^{-4} g/ml did have such an effect. The photoreflectance data shown in Figures 1 and 2 revealed a slow but definite darkening response (decrease in reflectance). While the response of *Rana pipiens* to the peptide, as demonstrated by the reflectance technique, is admittedly weak, it is at least as good as that reported for other chromatophore-stimulating substances such as cyclic AMP[9,10] and methylxanthines[6]. In contrast, *Anolis* skins responded maximally (Figure 4) to a concentration of 5×10^{-4} g/ml of the thermal polymer. This response was maximal and equivalent to that obtained with porcine B-MSH[11], and like MSH[7], could be reversed by norepinephrine.

Anolis skins were responsive to even lower concentrations (10^{-4}, 5×10^{-5} g/ml) of the thermal polymer, which caused a rapid near-maximal darkening of skins (Figure 5). Norepinephrine added to these skins resulted in a near 100% reversal of the polymer-induced darkening, and the subsequent addition of dichloroisoproterenol resulted in a further increased lightening of the skins. Melanin granule aggregation in response to catecholamine stimulation is mediated through α adrenergic receptors of the melanophores but is partially antagonized by a concomitant stimulation of β adrenergic receptors also contained by the melanophores[7]. Dichlorisoproterenol, a β adrenergic block-

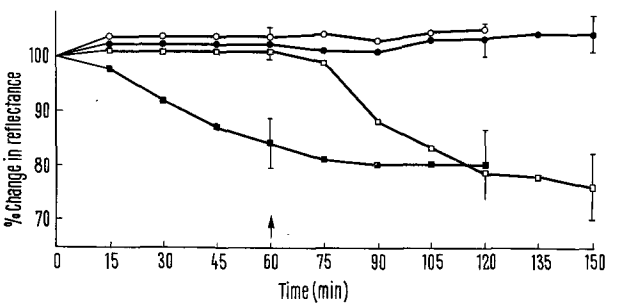

Fig. 3. In vitro response of *Rana pipiens* skins to a thermal polymer of amino acids possessing intermedin-like activity. 3 groups of skins were incubated in a Ringer solution of the polymer at a concentration of either 10^{-6} (□), 10^{-5} (●), or 10^{-4} (■) g/ml. At 60 min (arrow), a 5×10^{-4} g/ml solution of the polymer was substituted for the 10^{-6} g/ml (□) solution. One group (○) of the skins was maintained as a Ringer control. Results are means of the reflectance measurements from 8 skins per point on the graph.

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ing agent, blocks the agonistic effect of norepinephrine on the β receptor thus allowing a maximal melanin granule aggregating response resulting from an uninhibited α adrenergic receptor stimulation.

Discussion and conclusions. The observations made in this study indicate that the thermal polymer: arginyl, glutamyl, glycyl, histidyl, phenylalanyltryptophan not only stimulates melanin granule dispersion within melanophores but stimulates iridophore contraction as well. The combined effect of this material on both the iridophores and melanophores of *Rana pipiens* is responsible for the reflectance changes observed. While the decrease in reflectance of frog skins was admittedly weak it was definite and consistent and apparently of a slightly greater magnitude than that observed by Fox and Wang[4] in an in vivo method using the hypophysectomized frog[12].

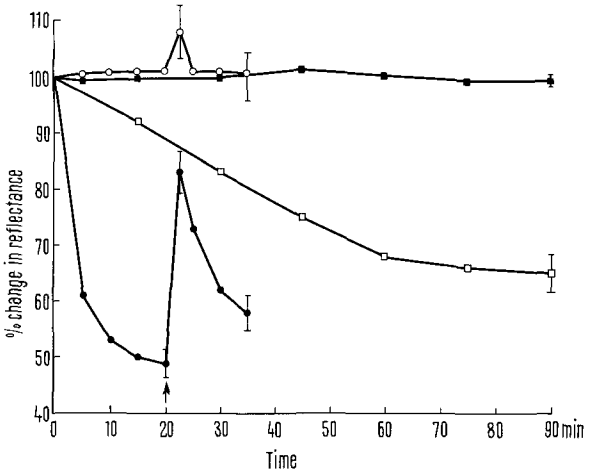

Fig. 4. Comparative in vitro response of *Rana pipiens* skins and *Anolis carolinensis* skins to a thermal polymer. One group of *Rana* (□) and one group of *Anolis* (●) skins were incubated in a 5×10^{-4} g/ml concentration of the peptide. One group of *Rana* (■) and one group of *Anolis* (○) skins were maintained as Ringer controls. At 20 min (arrow), norepinephrine ($10^{-5} M$) was added to both groups of *Anolis* skins. Results are means of the reflectance measurements from 8 skins per point on the graph.

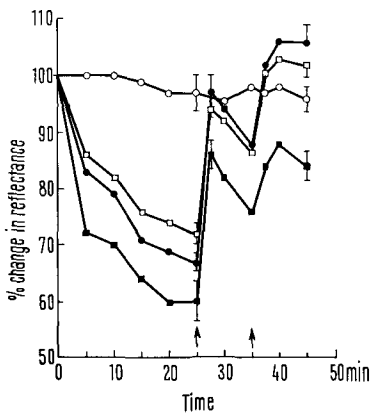

Fig. 5. Three groups of *Anolis* skins were darkened by a thermal polymer at one of the following concentrations: 10^{-5} (□), 5×10^{-5} (●), or 10^{-4} (■) g/ml. One group (○) of skins was maintained as a Ringer control. At 25 min (arrow), norepinephrine ($10^{-5} M$) was added to the 3 groups of darkened skins and this was followed at 40 min (arrow) by the addition of dichloroisoproterenol ($10^{-4} M$) to these same skins. Results are means of the reflectance measurements from 8 skins per point on the graph.

The experiments in which *Anolis* skins were used are of particular importance because the response of reptilian melanophores to the polymer is similar to their normal response to MSH. This response can be reversed by a normal mechanism of catecholamine stimulation involving α adrenergic receptor stimulation. This is the first demonstration that melanin granule dispersion in response to an MSH-like peptide can be reversed by another hormone. Apparently the mechanism of catecholamine antagonism of MSH which results in melanin granule aggregation is operative through the same small portion of the parent MSH molecule as is responsible for causing dispersion.

Of particular significance in these experiments is the demonstration that the chromatophores of *Rana* and *Anolis* respond differentially to the hexatonic polymer. Fox and Wang[4] point out that it is not merely the presence of a 6-component polymer which accounts for the chromatophore activity, but rather, there is a biological specificity attributable to the polymer used. It may be that *Anolis* melanophores respond more strongly to the polymer than *Rana* because the amino acid sequence of the thermal polymer resembles more closely that of the naturally occurring intermedin of *Anolis* than *Rana*. This is not unreasonable in view of the fact that it has been shown that *Anolis* has an intermedin which differs from that of mammals[12,13].

Zusammenfassung. Der Einfluss der Hitzepolymerisate Arginin, Glutaminsäure, Histidin, Phenylalanin und Tryptophan auf die Chromatophoren wurde geprüft. Die Reptilienhaut (*Anolis carolinensis*) zeigte auf Grund von photometrischen Reflexionsmessungen eine stärkere MSH-Reaktion gegenüber einem synthetischen kleinen Peptid als die Amphibienhaut (*Rana pipiens*). Die hautverdunkelnde Wirkung kann durch Noradrenalin und einen Blocker wieder wieder aufgehoben werden.

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[13] This study was supported by grant No. GB-8347 from the National Science Foundation.

Reversible Lenticular Opacities Induced in Rats by Emotional Stress

Adrenaline[1] or morphine type drugs[2] which release adrenaline from the adrenal medulla[3] have been found to produce acute reversible cataracts in rodents in conjunction with lack of lid reflex movements and exophthalmos. These lens opacities can be prevented by any drug which prevents the eyes being kept wide open[4] or by the closure of the eye[5]. This observation gave support to the idea that dehydration is the major stimulus of this type of