verschiedenem pH allein zeigten, dass schon eine Verminderung des Ausgangs-pH von 7,25 um mehr als 0,25 zu einer deutlichen Verkürzung der Gerinnungszeit führen. Die bei grösseren Histaminmengen beobachtete Verkürzung ist ausschliesslich durch die pH-Verschiebung bedingt.

*Diskussion.* Die vorliegenden, mit dem Standardtest der Heparin-Antithrombinwirkung durchgeführten Untersuchungen zeigen, dass Serotonin und Tryptamin in hohen Dosen eine heparinneutralisierende Wirkung ausüben, währenddem Histamin, Tyramin und Isoamylamin keinen Effekt haben. Die heparinneutralisierende Wirkung von Serotonin im System Cofaktor-Fibrinogen + Heparin + Thrombin ist, wenn auch bedeutend schwächer, analog derjenigen von Protamin (vgl.[5]). Die Untersuchungen geben damit neben den erwähnten, an isolierten Organen und *in vivo* erhobenen Befunden neue Anhaltspunkte für die Annahme, dass zwischen Serotonin und Heparin eine beide Stoffe neutralisierende Bindung zustande kommen kann. Die Frage, ob den vorliegenden Ergebnissen für den Ablauf der Gerinnung *in vivo* Bedeutung zukommt, bleibt offen.

R. KELLER

*Institut für Hygiene und Arbeitsphysiologie der ETH, Zürich, 14. Februar 1958.*

*Summary*

Investigations in the cofactor-fibrinogen system have shown that serotonin and tryptamine, although only in relatively high doses, reduce the antithrombin time prolonged by the action of heparin. Histamine has no such effect on the action of heparin, nor have tyramine and isoamylamine.

[5] R. MARBET, A. STUDER und A. WINTERSTEIN, *Thrombose und Embolie* (Benno Schwabe, Basel 1955), p. 362.

## On the Nature of Some Fluorescent Substances of Pterin Type in the Adult Skin of Toad, *Bufo vulgaris formosus*

In 1952, one of us[1] proposed the name Rana-chrome 1, 3, 4 and 5 for the fluorescent substances isolated from the skin of *Rana nigromaculata.* The aqueous solution of Rana-chrome 1, 3 or 5 shows a blue fluorescence, and the first two are transformed by irradiation or oxidation into Rana-chrome 5, 2-amino-4-hydroxypteridine-6-carboxylic acid. Rana-chrome 1 is found in most of the amphibians so far examined, and also in certain insects (e.g. *Drosophila, Bombyx*). This substance seems to be very likely the same as biopterin[2]. Rana-chrome 4 is identical with isoxanthopterin[3]. On the other hand, we[4] reported the presence of fluorescent substances of pterin type in the skin of toad: namely, small amounts of Rana-chrome 1, the complete absence of Rana-chrome 3, considerable amounts of Rana-chrome 5 and Bufo-chrome (= Bufo-chrome 1) presumably characteristic of *Bufo* species. These substances and riboflavin were found in considerable amounts in the colored part of the dorsal skin, while in the black spot of the ventral skin riboflavin alone was found. In the white or yellow part, very little of such substances occurred. The recent investigation revealed that the substance reported as Rana-chrome 1 in the previous paper was a mixture of two different components, the greater part of which differs from Rana-chrome 1 and belongs to the substance which we name 'Bufo-chrome 2'. The other component, Rana-chrome 1, exists only in traces and is rather difficult to recognize.

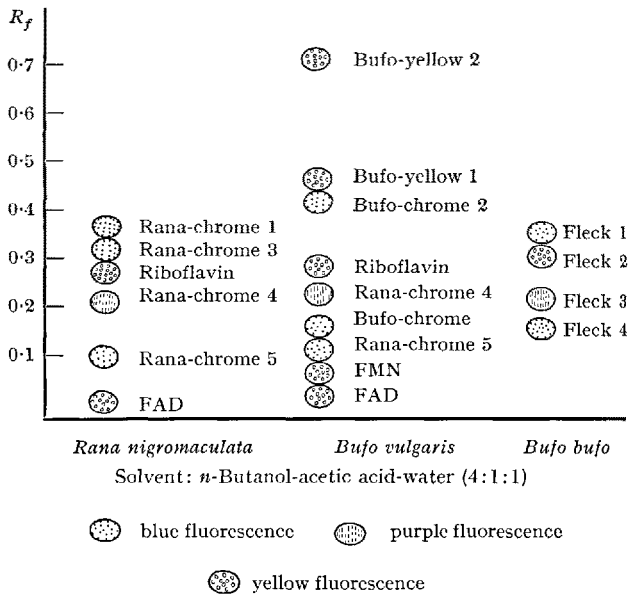


Fig. 1. – Chromatogram of fluorescent substances isolated from the dorsal skin of *Bufo vulgaris* and *Rana nigromaculata*, and that of *Bufo bufo* reported by ZIEGLER-GÜNDER[5].

The present paper deals with the nature of these two Bufo-chromes, together with two yellow substances isolated from the same material by the method of paper chromatography. Extraction was carried out by the slight modification of AWAPARA's method[6] or with 10% trichloracetic acid. The chromatogram is shown in Figure 1.

### *I. Bufo-chrome*

(a) Results on paper chromatography: The $R_f$ values of Bufo-chrome in various solvents differ from those of Rana-chrome 1 (Table I), although both behave chemically in a similar way (see below). (b) Effect of light: Photolysis of Bufo-chrome occurred fairly rapidly in the alkaline solution (pH 9·1) and rather slowly in the acidified medium (pH 2·5). The photolyzed product was determined as Rana-chrome 5 in respective cases. (c) Oxidation with $MnO_2$: The material treated was almost perfectly oxidized to Rana-chrome 5. (d) Ultra-violet absorption spectrum: Figure 2 shows the absorption spectra of Bufo-chrome in acid and alkaline solutions.

[1] T. HAMA, Zool. Mag. *61*, 89 (1952); Exper. *9*, 229 (1953). – T. HAMA and T. GOTO, C. R. Soc. Biol. *148*, 754 (1954). – T. HAMA, T. GOTO and K. KUSHIBIKI, C. R. Soc. Biol. *148*, 1313 (1954).

[2] E. L. PATTERSON *et al.*, J. Amer. chem. Soc. *77*, 3167 (1955); *78*, 5781, 5868, 5871 (1956). – H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. *77*, 4865 (1955). – M. VISCONTINI *et al.*, Helv. chim. Acta *38*, 397, 1222, 2034 (1955).

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*Table I*
$R_f$ values of Bufo-chrome, Rana-chrome 1, Bufo-chrome 2 and AHP

| Solvent | Bufo-chrome | Rana-chrome 1 | Bufo-chrome 2 | AHP |
|---|---|---|---|---|
| Butanol-acetic acid-water (4:1:1) . . . . | 0·17 | 0·35 | 0·42 | 0·42 |
| Butanol-ethanol-water (4:1:1) . . . . . | 0·07 | 0·24 | 0·29 | 0·29 |
| Propanol-1% ammonia (2:1) . . . . . | 0·22 | 0·50 | 0·45 | 0·45 |
| 5% acetic acid . . . . . | 0·75 | 0·80 | 0·72 | 0·72 |
| 5% sodium phosphate (secondary) . . . | 0·63 | 0·68 | 0·55 | 0·55 |

### *II. Bufo-Chrome 2*

(a) Results on paper chromatography: No difference was detected chromatographically between this substance and the synthetic 2-amino-4-hydroxypteridine (AHP)[7] (Table I). (b) Effect of light: The aqueous solution of Bufo-chrome 2 or AHP was exposed under ultraviolet light for 30 min. No significant change was observed on paper chromatogram in both cases. (c) Oxidation with $MnO_2$: This treatment caused no visible change of the material. (d) Ultraviolet absorption spectrum: The spectra in the alkaline ($n$/10 NaOH) and acid ($n$/10 HCl) media correspond to those of AHP. From these results, it may be safely concluded that the two substances, Bufo-chrome 2 and AHP, are identical.

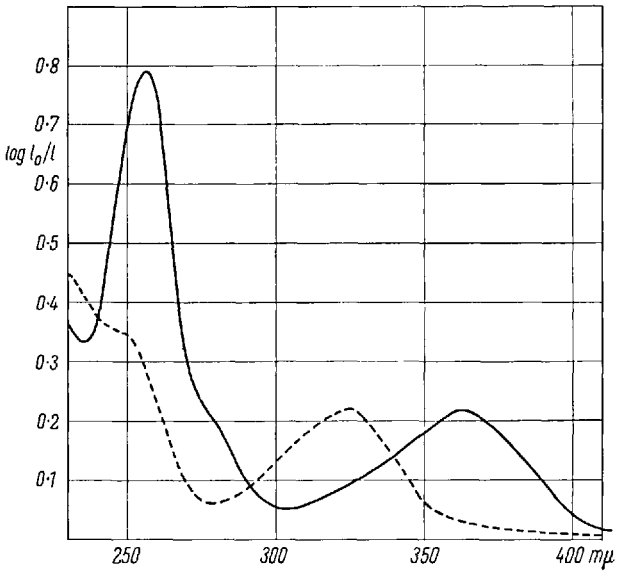


Fig. 2. – Ultraviolet absorption spectra of Bufo-chrome
——— $n$/10 HCl – – – – $n$/10 NaOH

### *III. Bufo-yellow 1 and 2*

(a) Results on paper chromatography: These two newly found yellow fluorescent substances do not occur in any great quantity. The $R_f$ values of these substances, riboflavin and rhacophoro-jaune[8], which was first found in the larval life of *Rhacophorus schlegelii arborea*, are shown in Table II. Rhacophoro-jaune is the same substance as the 'yellow pigment' isolated from *Drosophila 'se'* or *Bombyx 'lem'*[9]. (b) Effect of light and oxidation with $MnO_2$: These substances are thermostable. The ultraviolet irradiation or oxidation caused no significant changes except the considerable augmentation of the fluorescence in Bufo-yellow 1 after irradiation.

### *IV. Discussion and conclusion*

As a rule, in the adult skin of most amphibians AHP is scarcely found, whereas Rana-chrome 1 and isoxanthopterin exist in large amounts. However, in the skin of toad, the quantitative proportions of these substances are inverted. Bufo-chrome, which makes its appearance at the limb bud stage, is very abundant in the adult form. The presence of Bufo-yellow 1 has not yet been ascertained in other amphibians. Bufo-yellow 2 seems to be identical with the yellow substance found in the larva or adult of other amphibian species. Whether Bufo-yellow 1 or 2 is of pterin type remains to be shown.

[7] The authors express their acknowledgement to Prof. M. Akino (Tokyo Metropolitan University) who kindly donated the synthetic material.

[8] T. Hama et T. Goto, C. r. Soc. Biol. *149*, 859 (1955).

[9] H. S. Forrest and H. K. Mitchell, J. Amer. chem. Soc. *76*, 5658 (1954); *Ciba Foundation Sym. on Chemistry and Biology of Pteridines* (London 1954). – S. Nawa and T. Taira, Proc. Japan Acad. *30*, 632 (1954). – T. Goto and T. Hama, Zool. Mag. *66*, 92 (1957).

*Table II*
$R_f$ values of Bufo-yellow 1, 2, riboflavin and rhacophoro-jaune

| Solvent | Bufo-yellow 1 | Bufo-yellow 2 | Riboflavin | Rhacophoro-jaune |
|---|---|---|---|---|
| Butanol-acetic acid-water (4:1:1) . . . . | 0·45 | 0·66 | 0·28 | 0·38 |
| Propanol-1% ammonia (2:1) . . . . . | 0·44 | 0·75 | 0·42 | 0·49 |
| 3% urea . . . . . | 0·22 | 0·51 | 0·44 | 0·37 |
| 3% sodium citrate . . . . . | 0·14 | 0·40 | 0·39 | 0·30 |
| 5% acetic acid . . . . . | 0·30 | 0·65 | 0·48 | 0·45 |
| 3% ammonium chloride . . . . . | 0·18 | 0·42 | 0·43 | 0·35 |

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T. HAMA and M. OBIKA

*Biological Laboratory, Keio University, Yokohama-Hiyoshi (Japan), February 8, 1958.*

*Résumé*

A l'aide de techniques chromatographiques on a pu mettre en évidence dans la peau de *Bufo* adulte, outre plus de pigments fluorescents déjà connus, le bufo-chrome donnant de l'acide 2-amino-4-hydroxy-ptéridine-6-carboxylique par oxydation, le bufo-chrome 2 et deux substances à fluorescence jaune, le bufo-yellow 1 spécifique de l'espèce *Bufo* et le bufo-yellow 2 qu'on retrouve chez d'autres amphibiens.

## High Fat Diet and Mast Cell Count in Rat Mesenterium

The relationship between mast cells (heparinocytes), fat metabolism and atherosclerosis has attracted attention in recent years[1]. However, the question as to whether mast cells have a fundamental role in lipid metabolism still remains unanswered.

It was therefore of interest to ascertain whether a high fat diet would show any effect on the mast cell system.

Mast cell count in the mesenterium of rats maintained on diets with a varied fat content

| Group | Composition of diet | No. of animals | Average number of mast cells/animal |
|---|---|---|---|
| I. Low fat | 46 Cal.% protein<br>12 Cal.% fat<br>42 Cal.% carbohydrate | 20 | 1727·3*<br>S.D. = 319·08 |
| II. High fat | 13 Cal.% protein<br>80 Cal.% fat<br>7 Cal.% carbohydrate | 18 | 1572·4<br>S.D. = 315·05 |
| III. High fat + cholesterol | as in group II., however, 3% fat replaced by cholesterol | 19 | 1470·1*<br>S.D. = 234·6 |

* The difference between groups I and III significant for $P < 0{,}01$.

57 male Wistar rats, average weight 150 g, raised under identical conditions, were divided into 3 groups, each maintained on an isocaloric diet differing in lipid content (Table). All the rats had a normal growth curve. After 8 weeks, the rats were sacrified and their mesenterium was fixed in Schaffer's solution (2 parts 80% alcohol to one part 40% formaldehyde), and stained by the standard method with toluidine blue[2].

From the mesenterium of each animal three specimens were taken, always from corresponding *loci*. By means of an adapted occular, 50 square fields (each of 0,01225 sq. mm area) were examined at a magnification of 200 ×. The mast cell counts given in the table are averages from one animal (i.e. the number of mast cells from 150 fields, 50 fields from each specimen).

It can be seen that the high fat diet containing cholesterol produced a significant decrease in mast cell count after 8 weeks. A decrease, although not statistically significant, can be observed in the group kept on the high fat diet without the addition of cholesterol.

After completion of these experiments, GRUNBAUM *et al.*[3] published a report in which is apparent a small, statistically insignificant fall of mast cell count in the tissue of external ear of rats, fed a high fat diet.

A publication, cited above[4], has shown that there is a much lower mast cell count in the myocardium of atherosclerotics than in controls of the same age.

It is suggested that the effects of a high fat diet on the mast cell count is conditioned by loading and exhaustion of its secretory activity.

J. FODOR, P. FÁBRY, Z. LOJDA

*Institute for Cardiovascular Research, Institute of Human Nutrition, Physiological Laboratory, Institute of Embryology, Medical Faculty, Charles University, Prague (Czechoslovakia), February 12, 1958.*

*Zusammenfassung*

57 Ratten mit isokalorischer Diät und verschiedenem Fettgehalt wurden auf 3 Gruppen verteilt. Die Ratten der Gruppe mit Diät von hohem Fett- und Cholesterolgehalt zeigten eine statistisch signifikante Verminderung der Heparinocytenzahl in den Mesenterien.

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## On the Catechol Amine Levels in Blood Plasma after Stimulation of the Sympathoadrenal System

Improved techniques have made possible the chemical determination of adrenaline and noradrenaline in blood plasma. In the experiments described below such determinations were performed after stimulation of the sympathoadrenal system.

Cats of both sexes, weighing about 3·5 kg, were anesthetized with nembutal (25 to 50 mg/kg body weight intraperitoneally and later small intravenous doses when required). Carotid pressure was recorded using heparine as an anticoagulant. Injections were made in the jugular vein. Blood samples (18 ml) were taken through a polyethylene tubing in the femoral artery and collected in a 50 ml polyethylene bottle containing 2 ml of 1% disodium versenate in physiological saline. The bottle was kept in ice water during the collection of blood. The blood was immediately centrifuged at about 15000 × *g* for 10 min in a refrigerated International centrifuge. (Under these conditions no uptake of catechol amines in platelets was detectable.) The plasma was sucked off, and the blood corpuscles suspended in saline and reinjected. Perchloric acid extracts of plasma were neutralized by $K_2CO_3$. The precipitate was removed by centrifugation. The extracts