$10^{-3}M$ to $5 \times 10^{-6}M$. After incubation, the solutions were submitted to electrophoresis and immunoelectrophoresis as usual.

The radioactivity of the slides was estimated by means of a Geiger–Müller detector, and the dried agar-gel placed in direct contact with the emulsion of a Kodirex X-ray film. Exposure took place for varying lengths of time according to the activity measurements (generally 3 h to 6 days), whereupon the films were developed and the slides stained for proteins.

Results. The electrophoretic pattern of two normal leucocyte populations with about 80% neutrophilic granulocytes is seen in Figure 1. Five fractions are found in the cathodic area, two corresponding to the β-area and two to the γ-area of serum; the third γ-fraction is situated just cathodically to serum γ-globulin, and as a rule this is the most prominent fraction. In the anodic area three main fractions are seen. The two migrating faster than serum albumin contain nucleoprotein[8]; the third occupies the α-region. – The pattern of myeloid leukaemic leucocytes does not differ from the one described.

Esterase activity is seen both in the α- and γ-regions (Figure 2); some blurring of the active fractions takes place during the staining procedure because of diffusion of the proteins, as these are not fixed until after staining. In the γ-area two active fractions can be distinguished,

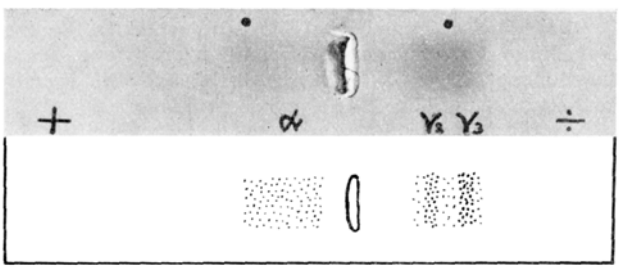

Fig. 2. Esterase activity in leucocyte extracts, revealed after electrophoresis.

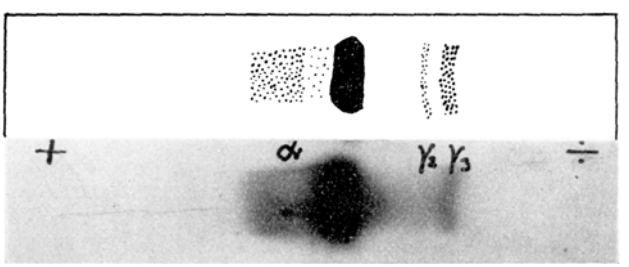

Fig. 3. Autoradiogram of leucocyte extracts incubated with DFP^{32} and submitted to electrophoresis.

corresponding to the γ-2 and γ-3 fractions of Figure 1. Both in the α- and γ-region, the activity was abolished in $10^{-3}M$ DFP and just distinguishable in a $2 \times 10^{-4}M$ solution.

In immunoelectrophoresis, two precipitation lines possess esterase activity, one in the α- and one in the β-γ-region.

Autoradiography of proteins incubated with DFP^{32} showed (Figure 3) blackening of the film in the α-area; in the cathodic part the γ-3 fraction was distinct, γ-2 faint. In immunoelectrophoresis, a distinct precipitation line was developed in the α-area, and a weaker bow in the β-γ-region corresponding to the ones possessing esterase activity; in a few preparations, a third very faint bow was seen in the anodic part of the γ-region.

Neither enzyme coloration nor autoradiography revealed differences between normal and leukaemic leucocytes.

Discussion. Diisopropylfluorophosphate inhibits esterases and certain other enzymes, being irreversibly bound to the hydroxyl group of a serine residue[10] with liberation of hydrogen fluoride. The diisopropylphosphate liberated by the degradation of the labelled proteins does not react with proteins. DFP^{32} is thus suitable for the labelling of cells, although this labelling means inhibition of several important cellular enzymes. As regards erythrocytes, the life span as measured by DFP^{32} corresponds to the values found by other methods.

In our electrophoretic studies on leucocyte proteins, we have found close agreement between esterase activity as visualized by a staining reaction in the agar-gel and DFP^{32} binding capacity of the leucocyte extracts as revealed by autoradiography. That the main activity of each of the three active fractions visualized by agar-gel electrophoresis is due to one immunologically homogeneous protein is suggested by the immunoelectrophoretic results; two precipitation bows possess esterase activity, and three are labelled with DFP, their centers being in the α-, late β-, and γ-area, respectively, corresponding to the fractions obtained by simple electrophoresis.

Résumé. Après électrophorèse en gélose des protéines extraites de leucocytes humains, l'activité estérasique des fractions fut comparée à leur affinité pour DFP^{32}. Les deux méthodes montraient trois fractions actives, tant en leucocytes normaux qu'en leucocytes de leucémie myéloïde.

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The Effect of Inhalatory Adaptation to Histamine on Histamine Shock and the Anaphylactic Shock

The effect of adaptation to histamine (administered in the form of aerosols) on the course of histamine shock and of the anaphylactic shock has been studied in guinea-pigs.

There are few data concerning the influence of adaptation to histamine on the course of the anaphylactic shock[1]. No data were found in the bibliography as to the influence of adaptation produced by a prolonged administration of histamine aerosols on the course of the anaphylactic shock. The effect of adaptation to histamine, as produced by different methods, on the course of hista-

[1] E. SMITH-KARÁDY, J. Immunol. *41*, 1 (1941).

mine intoxication was studied more extensively. The results of experiments are, however, incompatible[2-6].

Methods. Experiments were carried out on male guinea-pigs, 300–400 g in weight. Animals were adapted to histamine for 4–6 weeks, by daily exposure to 0.6% histamine aerosol until a high degree of tolerance was obtained, i.e. until animals, in which the initial time of exposure was 1–2 min, could be kept in histamine aerosol for 20 min without pathological signs. Methodological details of the procedure are described elsewhere[6]. A D-30 aerosol generator was used for producing histamine aerosols[7]. Sensitization of animals and anaphylactic shock were produced by the method described by HERBERTS[9], using hen's egg albumin. Lethal dose of histamine (0.66 mg/kg intravenously of histamine dihydrochloride per kg of body weight) was accepted on the basis of our previous studies[10].

Results. The results of experiments on the course of the anaphylactic shock in animals adapted to histamine are presented in Table I. The results of experiments on histamine tolerance in animals adapted to this amine are presented in Table II.

Table I. The course of the anaphylactic shock in guinea-pigs adapted to histamine

Group	Number of animals	Survived	Died
C	23	2	21
C + H_e	10	2	8
A	29	7	22
A + H_e	15	14	1

Table of statistical significance

Group	C + H_e	A	A + H_e
C	$0.7 < p < 0.8$	$0.2 < p < 0.3$	$p < 0.01$
C + H_e		$0.9 < p < 0.95$	$p < 0.01$
A			$p < 0.01$

Abbreviations: C, control group; sensitized animals, anaphylactic shock produced. C + H_e, control group; exposure to histamine aerosol immediately before shock-producing dose of antigen. A, adapted to histamine. A + H_e, adapted to histamine; additional exposure to histamine aerosol before shock-producing dose of antigen.

Table II. Histamine tolerance in the adapted animals

Group	Number of animals	Survived	Died
A + H_e	6	6	0
A	5	0	5
C + H_e	5	0	5
A + H 0.33 mg/kg i.v. 15 min H 0.66 mg/kg i.v.	8	8	0
C + H 0.33 mg/kg i.v. 15 min H 0.66 mg/kg i.v.	5	1	4

Abbreviations: A + H_e, adaptation to histamine; additional exposure to histamine aerosol before lethal dose of histamine. A, adaptation to histamine. C + H_e, controls; additional exposure to histamine aerosol before lethal dose of histamine. A + H 0.33 mg/kg i.v., adaptation to histamine; intravenous injection of 0.33 mg/kg of histamine before its lethal dose. C + H 0.33 mg/kg i.v., controls; intravenous injection of 0.33 mg/kg of histamine before its lethal dose.

Discussion. The data just presented indicate that there is a parallelism in the course of histamine intoxication and anaphylactic shock in animals adapted to histamine by the inhalatory route. In both cases, histamine-adapted animals showed increased resistance only when they were subjected to an additional exposure to histamine aerosols immediately before the lethal histamine dose or shock-producing dose of antigen, or when they were given half the lethal histamine dose. Sensitivity of adapted animals is only slightly lower than that of the controls when the lethal dose of histamine or shock-producing dose of antigen were administered 24 h after the last exposure to histamine aerosol. It should be added that single exposure of control animals did not alter their sensitivity to histamine and anaphylactic shock.

It can be concluded from the above observation that, during adaptation to histamine, the resistance to histamine and to the antigen-antibody reaction products is increased only in adapted animals which were exposed to contact with histamine immediately before the lethal histamine dose or shock-producing dose of antigen. No increase in resistance was observed in non-adapted animals treated in the same way. This would indicate that the decrease in resistance is not related to tachyphylaxy phenomenon.

As might be suggested from the above results, in the adapted guinea-pigs there is a 'potential ability' to tolerate big doses of exo- and endogenous histamine.

This ability comes to light by additional exposure to aerosol or by intravenous injection of small histamine amounts. Body reserves which are gained in the course of adaptation would be 'mobilized' by exposure to aerosol or by histamine injection to the adapted animals. By this 'mobilization', the organism is protected against histamine death.

Résumé. Les animaux adaptés à l'histamine sont moins sensible à l'histamine et aux produits d'une réaction antigène-anticorps. La tolérance des cobayes adaptés est augmentée particulièrement après une exposition supplémentaire dans l'aérosol histaminique.

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[7] The D-30 aerosol generator, designed by DAUTREBANDE[8], permits the production of a homogenous aerosol with its particles 0.2 μ in diameter. The instrument was obtained from Dr. J. S. SCANLAN, Richardson-Merrell Inc., New York, to whom we with to offer our grateful thanks.

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