Summary

Certain features of a modification of WOOLFE and MACDONALD algesimetric method are outlined.

Systematic variations of the mean reaction times occur independently of every manipulation other than the exposition of mice to thermoalgesic stimuli. The principal ones are:

(1) The second determination yields a mean value which is always superior to that of the first one.

(2) Repeated determinations yield values which become stable only when the frequency of the expositions is relatively low (one every 15 min or less); with high frequency exposures (group of three exposures every 30 or 120 min) the mean reaction time increases considerably.

These variations still appear after adrenalectomy. Practical conditions for the use of this method concerning the study of analgesics are deduced from these facts and an experimental scheme is given as an illustration.

A Humoral Agent with Immediate Eosinopenic Effect other than Cortisone

Fluctuations in the number of circulating eosinophils have recently been the subject of numerous papers in connection with the different states of "stress-conditions". The literature supports the statement that activation of the non-specific defence mechanism is regularly followed about 4 h later by a pronounced diminution of circulating eosinophils. In the so-called Thorn-test[1], a decrease of eosinophils of more than 50% indicates a sufficient reserve of the hypophyseo-adrenal axis. Authors generally assume that cortisone-like substances liberated from the adrenals during different injuries (adrenaline, formaline, salicylate, etc.) are responsible for the eosinopenic effect. This statement was based on the well known fact that only cortisone and hydrocortisone are capable of producing significant eosinopenia in adrenalectomised animals. Cortisone therefore was regarded as the end-substance of the above mechanism.

Some controversial data, however, were published in the past. According to ROMANI[2], the mechanism of eosinopenia induced by cortisone and A.C.T.H. differs from that of formaline and adrenaline. OLÁH and VARRÓ[3] demonstrated that the cortisone eosinopenia fails to develop in deep hexobarbitone narcosis.

It is a well-known fact that 3–4 h are necessary for the development of a significant fall of eosinophils, whatever kind of stressors were used—even if the suspected end-substance, cortisone, was administered. It is interesting in this relation that LOVE[4] succeeded in producing eosinopenic response in animals subjected to adrenalectomy as soon as 10 min after stress. It seems therefore that sufficient amounts of cortisone are liberated during this short period, and the subsequent time is needed for developing the eosinopenic effect of cortisone. The conception of a direct effect of cortisone is finally weakened by the fact that up to the present no one has been able to demonstrate irrefutably its *in-vitro* eosinolytic power.

We therefore supposed that cortisone induces eosinopenia by mobilizing some hitherto unidentified substance.

Experiments.—Experiments were carried out on albino rats weighing 100–180 g. Eosinophil counts were taken by the modified Dunger-Method[1]. Rats were stressed by 50 µg subcutaneous adrenaline. At the time of eosinophil depression blood was withdrawn from the (decapitated) donators. Serum was isolated within 30 min and 0·5–0·75 ml was injected in the tail vein of acceptor rats. Eosinophils were counted immediately before and 10, 20, 30 min after the serum was injected.

Control animals received sera of non-stressed rats under similar experimental conditions.

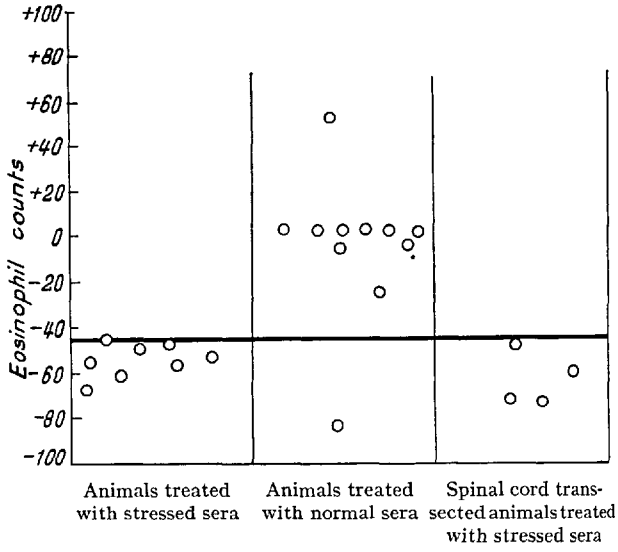

Fig. 1.—Percent changes of the circulating eosinophils of rats in the first 30 min after different serum-transfusions.

Results are presented in Figure 1. As demonstrated, sera of stressed animals regularly induced significant eosinopenia within 10–30 min. This phenomenon failed to develope in rats transfused with non-stressed sera.

Absolute eosinophil count of rats after intravenous administration of Cortone-Merck.

No.	Microgram dose	Eosinophil counts				
		Control	After the Cortisone injection			
			10 min	20 min	30 min	4 h
1	125	1280	3500		2740	216
2	125	885	2350	1370	1090	
3	62·5	760	1105	510	516	55
4	62·5	1010	1175	1010	922	
5	50	378	415	333	333	

In further experiments we administered 62·5–125 µg cortisone (Cortone-Merck was diluted with physiological saline 1:400–1:200, and 1 ml was injected intravenously). Eosinophils were counted before and 10, 20, 30 min after the injection. To control the preparation in two cases the 4 h eosinophil count was also determined.

As is shown in the Table intravenous cortisone fails to produce a significant eosinopenia within 30 min. On the contrary a moderate eosinophilia is the rule.

[1] G. W. THORN *et al.* J. Amer. Med. Asoc. *137*, 1005, 1544 (1948).

[2] J. P. ROMANI, C. r. Soc. Biol. *146*, 654 (1952).

[3] F. OLÁH, V. VARRÓ, M. MAJOROS, K. KOVÁCS, and D. BACHRACH, Orvosi Hetilap *92*, 1129 (1951).

[4] W. D. LOVE, Proc. Soc. exp. Biol. Med. *75*, 639 (1950).

[1] A. DUNGER, Münch. med. Wschr. *57*, 1942 (1910). – I. RECANT *et al.*, J. Clin. Endocrin. *10*, 187 (1950).

We investigated further the role of autonomic nervous pathways in the production of the transmissible immediate eosinopenia. The thoracic part of the spinal cord (between D_1–D_2) of acceptor rats was transsected. On the second day transfusions were carried out using stressed and normal sera under conditions described above.

As is shown in Figure 1, the transsection of the spinal cord does not alter the transmissible eosinopenia.

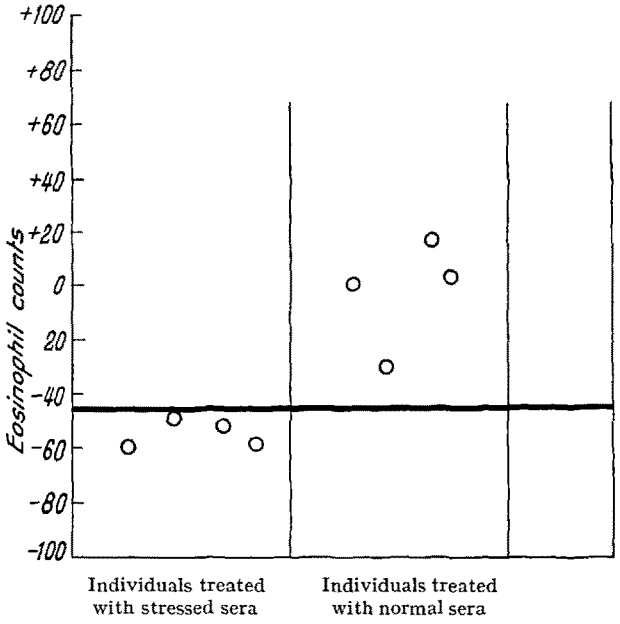

Fig. 2.—Percent changes of the circulating eosinophils of humans in the first 30 min after different serum-transfusions.

Finally we performed similar experiments in humans. The donator was treated with 400 μg adrenaline s.c. and after the development of a significant eosinopenia (the Thorn-test was performed in every case) 300–350 ml blood was withdrawn under sterile conditions. Blood clotting was prevented by physiological citrate solution (1:10) and plasma was isolated by centrifuging. We then injected 100–140 ml of (isolated) plasma into acceptor-patients, free from any demonstrable hypophyseo-adrenal lesion. The eosinophil counts of acceptors were determined as in the animal experiments. Control experiments were carried out on the same persons with the plasma of non-stressed subjects.

Figure 2 shows that, as in our rat-experiments, a significant eosinopenia develops within 10–30 min in persons receiving stressed plasma, while no change occurs in the controls.

Discussion.—We demonstrated that sera deriving from individuals stressed with adrenaline 4 h before possess the capacity to produce significant eosinopenia *within 30 min* if transfused to normal subjects.

This phenomenon suggests the circulation of a substance during eosinopenia, being capable of evoking an immediate "eosinopenic" action, in contrary to cortisone.

Although it is very unlikely that cortisone has any direct effect on eosinophils, this possibility cannot be ruled out by our present experiments.

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Zusammenfassung

Es wird gezeigt, dass Serum aus adrenalinvorbehandelten Ratten, vier Stunden nach Adrenalininjektion in normale Tiere transfundiert, bereits nach 30 min eine mehr als 50%ige Eosinopenie verursacht. Das Auftreten eines humoralen Wirkstoffes während der Eosinopenie wird diskutiert und mit Cortison verglichen.

Sensibilité du spermophile *(Citellus fluvus concolor)* à *Mycobacterium tuberculosis* var. *bovis*-B.C.G.

Nous avons découvert la sensibilité du hamster (*Mesocricetus auratus*) au bacille B.C.G.[1], et continuant nos essais, nous avons confirmé que les passages en série de l'infection étaient possibles chez le hamster soit par inoculation d'organes de hamsters morts, soit par inoculation de cultures provenant de ces organes. Au cours des passages chez *Mesocricetus auratus*, les inoculations systématiques d'organes ou de cultures provenant de ces organes à des cobayes, ont confirmé, dans la limite de nos expériences, l'absence de pouvoir pathogène du bacille B.C.G. pour cet animal. Enfin, nous avons constaté que des doses de bacilles B.C.G. très inférieures à celles utilisées dans nos premières expériences (1 mg par exemple) étaient capables de tuer le hamster après inoculation intra-péritonéale.

Nous nous sommes demandés si d'autres rongeurs possédaient une sensibilité semblable à celle du hamster. Nous avons constaté que le spermophile (*Citellus fulvus concolor*) était, lui aussi, sensible au bacille B.C.G.[2].

Trois spermophiles ont été inoculés par voie intra-péritonéale avec 150 mg de bacilles B.C.G., trois par la même voie avec 100 mg et trois avec 50 mg. Huit de ces animaux sont morts dans des délais variant entre deux et quatre mois, sans qu'il paraisse y avoir de rapport entre la dose inoculée et le temps de survie. Un est encore vivant. A l'autopsie on constate une hypertrophie de la rate sans tubercules visibles, les autres organes paraissant normaux à l'examen macroscopique. Le foie, la rate, les reins, les poumons contiennent des quantités considérables de bacilles acido-alcoolo-résistants. Le spermophile est bien entendu sensible au bacille tuberculeux humain ou bovin par voie sous-cutanée à des doses de 0,1 mg. La mort survient alors en un à deux mois. A l'autopsie on constate souvent des tubercules visibles macroscopiquement sur la rate, le foie, le poumon. On peut trouver des ganglions grossis et caséifiés. Les organes contiennent des quantités assez grandes de bacilles acido-alcoolo-résistants, moins importantes peut-être que celles observées chez les animaux inoculés avec le bacille B.C.G.

Cette sensibilité du spermophile n'a pour nous aucun rapport avec la valeur immunisante du bacille B.C.G. contre la tuberculose de l'homme. Elle n'est que la traduction d'une sensibilité particulière d'une espèce animale.

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Institut d'hygiène, Université de Lausanne, le 26 mars 1953.

[1] P. Hauduroy et W. Rosset, C. r. Acad. Sci. *232*, 445 (1951).

[2] Ces rongeurs nous ont été procurés par M. le docteur Baltazard, Directeur de l'Institut Pasteur de Téhéran, que nous tenons à remercier très vivement de son obligeance.