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Investigations on Some Experimental Inflammations in Mouse Paws

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For laboratory evaluation of antiphlogistics with reference to their action on inflammatory edemas the rat paw edema is the most frequently used inflammation model. One way of determining the intensity of the edema was the continuous plethysmometry of the paw (*Wilhelmi and Domenjoz*, 1951), a method which required anesthesia. However, it was shown by *Domenjoz* et al. (1957) that the results, e.g. the antiphlogistic action of salicylates, were affected by anesthesia. Therefore, experiments on the unanesthetized rat and e.g. weighing of the amputated paws (*Halpern and Briot*, 1950) is now one of the methods of choice.

These evaluations are currently done with rats, the mouse being used only by a few authors. Very often it would be desirable to work with mice instead of rats, the mouse requiring lesser quantities of testing material. We therefore examined whether the mouse was useful for the evaluation of antiphlogistics by studying its response to various phlogistics and by transferring to this species the routine method currently used with the rat.

Cooper, Schmidt and Schou (1957) used silver nitrate, turpentine and compound 48/80 as phlogistics injected into the planta pedis in a volume of 0.02 ml. After development of the edema the antero-posterior diameter of the paws was determined with a microscope with an ocular scale. *Northover and Subramanian* (1961 and 1962) studied edemas produced by subcutaneous injections (0.05 ml) of formalin and serotonin into the dorsum of the mice paws. Prior to the injection of the phlogistic the animals received an intravenous injection of "Evans blue", so that swelling and blueing could be noted separately by subjectively assigned scores. The uninflamed paws serving as controls were injected with saline. *De Salva and Evans* (1962) used turpentine inflammation (0.006 ml of undiluted turpentine). The amputated paws were weighed and the results expressed as percentage paw weight increase/20 g mouse by means of the following equation $\frac{L-R}{R} \times \frac{BW}{20\,g} \times 10^2 = \%$ increase, wherein L and R are the weights of the left and right paws and BW is the body weight of the mouse.

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Methods

Male white mice of the strain NMRI were used. The animals were allowed free access to water and food during the whole experiment. The inflammatory agent was injected subcutaneously in the planta pedis of the left hind paw.

The following substances were tested:

1. *Phlogistics:* formalin 3, 6 and 10%, silver nitrate 1%, baker's yeast 10%, dextran 6%, egg white 20%, kaolin 10%, histamine diphosphate 0.2 and 0.5%, serotonin 0.005, 0.025, 0.05, and 0.25%. Of these solutions 0.02 ml was injected. Furthermore, hyaluronidase (Kinetin®, Schering) was examined in the following doses 0.2 u./0.02 ml, 1 u./0.06 ml and 1 u./0.02 ml (*Dewes*, 1955). Solutions and suspensions were prepared with saline except in the case of silver nitrate where distilled water was used.

2. *Preparations tested with regard to inhibition of swelling:*

a) *Miscellaneous drugs.* Iproniazide phosphate, morphine hydrochloride, chlorpromazine (Largactil®, Specia), chloropyramine (Synpen®, Geigy), phentolamine (Regitin®, Ciba), calcium gluconate (Calcium "Thilo"), lysergic acid diethylamide (LSD), cortisone acetate (Cortison, Ciba). Solutions and suspensions were prepared with saline.

b) *Antipyretics-analgetics.* Quinine hydrochloride, aminopyrine, phenazone (antipyrine), sodium-salicylate, all dissolved in distilled water—phenylbutazone (Butazolidin®, Geigy), oxyphenbutazone (Tanderil®, Geigy), cinchophen, all dissolved in distilled water by addition of sodium hydroxide—acetylsalicylic acid dissolved in distilled water by addition of sodium bicarbonate.

For the evaluation of the degree of swelling the animals were killed by crushing the neck and both the hind paws were amputated at the tarsocrural joint and weighed separately. The weights were noted as the nearest whole multipla of 5 mg. The percentual increase in weight of the inflamed paw in relation to the control paw was then calculated for each pair of paws and the mean values were treated statistically by conventional methods. Generally groups of 10 mice were used. Further data are given below. When antiphlogistics were tested a control experiment was carried out every day as experience has shown that the degree of swelling can vary a great deal from one day to another, a phenomenon which also has been stated by other authors (*Haberland*, 1959).

Results

I. *Investigations concerning the qualification of the method.* One of the first questions arising by the direct application to mice of the standard method used in rats is whether the amputation can be performed with sufficient accuracy. Table I shows the results of an experiment where the paws of 36 untreated mice were amputated and weighed with the accuracy of 1 mg. As an un-

treated paw generally has a weight between 100 and 130 mg it can be seen from the table that the error of the amputation technique will hardly exceed ± 6%.

TABLE I

Distribution of the weight difference between left and right paw in untreated mice

	Left paw biggest							Right paw biggest						
Difference in weight (mg)	6	5	4	3	2	1	0	1	2	3	4	5	6	
Number of animals	1	1	0	1	2	8	9	8	2	2	2	0	0	
Total	13						9	14						= 36

To explain the relationship between differences in body weight within a single group of test animals (weight class latitude) and the error of the mean value of the responses a special experiment was carried out as illustrated in Table II. We found that the weight of the untreated paws increased linearly with the body weight in accordance with the findings of *de Salva and Evans* (1962) but we also observed that the absolute weights of the edemas increased with increasing body weights. From Table II it can be seen that both the degree of swelling and the mean error of the mean value are extensively independent of the dispersion of the body weight. The adjustment of the results by means of the equation mentioned above (*de Salva and Evans,* 1962) must therefore be regarded as unjustified as it implies that the absolute values of the differences in weight between the two paws are constant. Furthermore, Table II shows that the percentual mean error does not exceed 10%,

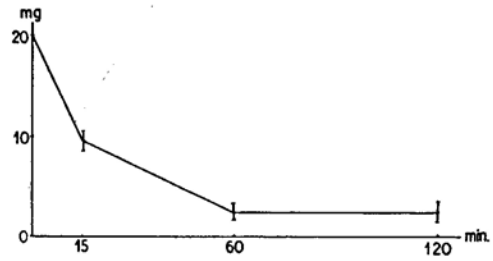

Fig. 1. Resorption of injected saline from the mouse paw. The values given represent the average of 10 values ± mean error.

which can be regarded as satisfactory. Finally, we tried whether the injection of 0.02 ml saline was able to induce a tissue reaction forming an edema. The result is shown in Fig. 1. Three groups of ten mice each were injected with sterile saline and the paws were amputated after 15, 60 and 120 minutes and weighed. It appears from the figure that after 15 minutes approximately half of the injected amount had disappeared. After one hour the difference in weight between the treated and the untreated paws is not significant. However, it is difficult to say how the resorption is influenced by a simultaneously developing inflammation. So we consistently subtracted 20 mg (the amount injected) from the weight of the inflammed paw, and we also omitted to inject the control paws with saline.

TABLE II

Relationship between weight class latitude, swelling and mean error
The inflammations were induced by injection of a 10% solution of formalin (3.5% formaldehyde) and the paws were amputated 2 hours later

Number of animals	Body weight g	Interval g	% Swelling	Mean error	Mean error in %
9	22	0	39	± 2.5	± 6.4
5	21	2	36	± 1.7	± 4.7
4	23				
5	20	4	32	± 1.9	± 5.9
4	24				
4	19	6	46	± 3.0	± 6.5
5	25				
5	18	8	42	± 2.4	± 5.7
5	26				
5	17	11	38	± 2.7	± 7.1
5	28				
5	14	16	41	± 2.4	± 5.9
5	30				

II. *Experiments with phlogistics.* We studied the sensitivity of mice to different phlogistics as mentioned above. The results are summarized in Table III, from which it appears that serotonin, formalin, silver nitrate, and yeast are useful as phlogistics in the

mouse. The dextran edema is generally considered to be a phenomenon specific for the rat (*Morrison, Richardson and Bloom,* 1951) and in fact we could not observe any reaction to injections of dextran in the mouse. Hyaluronidase also failed to produce any edema. In the rat hyaluronidase induces a fast occurring and large swelling but this reaction too seems to be species specific. Possibly it does not even depend on the hyaluronidase itself but on traces of impurities (*Benditt* et al., 1951). In the same way injections of egg white were tolerated without any inflammatory reaction. Histamine causes only a small and short lasting edema. The maximal effect (15-20% swelling) occurs after approximately ten minutes and after thirty minutes the swelling had almost disappeared.

TABLE III

Rate and intensity of the swelling after a series of phlogistics

Substance	Concentration %	Swelling max. after:	Swelling intensities obtained in %
Serotonin	0.005	30 min.	24
	0.025–0.05–0.25		33–44
Silver nitrate	1	2 hours	48–61
Yeast	10	3 hours	56–79
Kaolin	10	24 hours	31
	3		11–31
Formalin	6	2 hours	24–38
	10		32–56
Dextran	6	—	—
Egg white	20	—	—
Histamine diphosphate	0.2–0.5	10 min.	14–18
Hyaluronidase *		—	—

* Injected doses: 0.2 u./0.02 ml, 1 u./0.06 ml, and 1 u./0.02 ml.

III. *Experiments with antiphlogistics.* This group of experiments was divided into two series. In the first series a number of substances with more or less specific anti-inflammatory activity was tested. (See "miscellaneous drugs" above). The substances were injected intraperitoneally 15 minutes before the injection of the phlogistic. However, cortisone acetate was injected as a suspension subcutaneously five and three hours before the inflammation was provoked. The results of these experiments are summarized

TABLE IV

Inhibition of some artificial inflammations in the mouse paw

$t > 2.10$ gives the probability level $P < 0.05$
$t > 2.88$ gives the probability level $P < 0.01$

		Serotonin 0.025%		Formalin 10%		Silver nitrate 1%		Yeast 10%	
		Inhibition	t	Inhibition	t	Inhibition	t	Inhibition	t
Iproniazide phosphate	200 mg/kg i.p.	23%	3.0	16%	1.9	16%	2.7	22%	4.2
Morphine chloride	10 mg/kg i.p.	0%		33%	4.2	20%	3.2	25%	4.2
Chlorpromazine	5 mg/kg i.p.	42%	5.0	19%	2.3	18%	2.8	52%	11.0
Chloropyramine	20 mg/kg i.p.	50%	5.5	0%		0%		25%	4.1
Phentolamine	5 mg/kg i.p.	14%	1.5	0%		0%		11%	2.4
Calcium gluconate	500 mg/kg i.p.	0%		0%		0%		25%	5.5
LSD	0.1 mg/kg i.p.	36%	4.0	0%		0%		19%	3.6
Cortisone acetate	2 × 6 mg/kg s.c.	56%	11.0	11%	1.4	—		13%	1.9
Cortisone acetate	2 × 50 mg/kg s.c.	37%	6.0	19%	3.3	—		29%	3.9

TABLE V

Inhibition by some analgetics-antipyretics of artificial inflammations in the mouse paw

$t > 2.10$ gives the probability level $P < 0.05$

$t > 2.88$ gives the probability level $P < 0.01$

Interval		Yeast 10%				Formalin 10%				Serotonin 0.025%			
		½ hour		2 hours		½ hour		2 hours		2½ hour		4 hours	
		Inhibition	t	Inhibition	t	Inhibition	t	Inhibition	t	Inhibition	t	Inhibition	t
Aminopyrine	170 mg/kg p.o.			29%	5.1	30%	4.3	10% *	1.3	34%	5.8		
Phenazone	430 mg/kg p.o.	27% *	3.8	16% *	2.3	21%	2.7	11%	1.2	36%	6.3		
Phenylbutazone	125 mg/kg p.o.			30%	5.1			20%	3.3	16%	2.6	10%	1.5
Oxyphenbutazone	50 mg/kg p.o.			19%	3.3			20%	3.1	7%	1.4	11% *	1.2
Acetylsalicylic acid	675 mg/kg p.o.			25%	4.2			31%	4.7	25%	3.5		
Sodium salicylate	680 mg/kg p.o.	42%	6.0	25% *	3.8	32%	4.2	16%	2.6	25%	3.5		
Quinine chloride	90 mg/kg p.o.	0% *		6%	1.3	19%	2.6	0% *		36%	6.5		
Cinchophene	375 mg/kg p.o.			32%	6.7			21%	3.3	7%	1.1	11% *	1.5

* These results are from repeated experiments where the first experiments gave the result 0%.

in Table IV from which it appears that the formalin and the silver nitrate edemas react fairly uniformly so that it is very likely that they involve similar mechanisms in distinction to the serotonin and yeast reactions. Only the yeast edema was inhibited by all the substances tested.

It has been shown that two locally isolated but simultaneous inflammations in the same animal counteract each other. (*Domenjoz, Theobald and Mörsdorf,* 1955; *Büch and Wagner-Jauregg, 1962).* If the antiphlogistic is administered intraperitoneally the possibility must be considered that this traumatism within the abdominal cavity might influence the inflammation induced in the paw. In our experiments, however, we saw a rather distinct specificity in the action of the tested antagonists against the different inflammation models and for that reason we consider this explanation as unlikely in the present case.

The aim of our other series of experiments was to compare the antiphlogistic activities of some known substances from the group of antipyretics-analgetics. In order to obtain some sort of biological equivalency of the applied doses these agents were given per os in doses corresponding to the LD 50 by intravenous application. When the inflammation was provoked by formalin or yeast the compounds tested were given two hours before the injection of the phlogistic. In the case of serotonin they were given two and a half hours before with regard to the shorter time of development for this edema. In those cases where the responses were small or absent new experiments were carried out in which the time between the application of the test substance and the phlogistic was changed to half an hour with yeast and formalin and to four hours with serotonin. In this group of experiments, too, the animals were allowed free access to food and water, partly to avoid a possible "hunger stress", and partly to reduce a possible local irritation of the stomach.

The results are summarized in Table V. Even with this arrangement of doses one must expect considerable variations in the results, partly due to the different rates of resorption, biotransformation, and elimination, and partly to the expected difference between the therapeutic index of the substances. However, it appears from the table that the order of magnitude of nearly all the significant inhibitions is between 20-35%, which seems to justify this kind of standardisation.

Discussion and Conclusion

On the basis of the results obtained it is difficult to decide whether the biological mechanisms of the inflammation models mentioned above are identical with the corresponding mechanisms in the rat. However, some few points of interest should be stressed. The edema caused by yeast is probably due to polysaccharides and one could therefore expect to find some similarity to the dextran reaction in the rat. It has been shown that sodium salicylate (500 mg/kg sc.) has excellent effect on the edema induced by dextran in the rat, while the formalin-induced edema is affected by sodium salicylate to a lesser degree (*Domenjoz* et al., 1957). From Table V it can be seen that the yeast edema in the mouse is particularly susceptible to salicylate. The formalin edema is also significantly depressed by salicylate but to a smaller extent. The latter finding is in agreement with *Northover and Subramanian* (1962). *Gross* (1950) showed that antihistaminics (pyribenzamine, phenergan) did not influence the formalin edema in the rat and *Northover and Subramanian* (1962) did not find any effect of mepyramine maleate in the mouse. These findings are confirmed by our results with chloropyramine. It is remarkable that quinine was the only substance having no effect on the yeast edema in the mouse. Finally, it should be pointed out that in contrast to *Northover and Subramanian* (1962) we found oxyphenbutazone active against the formalin edema.

As a result of these investigations it may be concluded that mice to the same extent as rats are useful for the screening of antiphlogistics. As the inflammation models mentioned above can be considered as being different by their mechanisms, it is recommended to use at least two different types for screening purposes. In the same manner as in the case of rats it is impossible to make standard directions for the dosage and the way of application as these data in every single case depend on the toxicity, resorption rate, local irritating properties, etc. of the substances investigated. The results obtained with mice should be treated with the same criticism as those obtained with rats.

Summary

Some different phlogistics have been tested in the mouse paw. Serotonin, formalin, silver nitrate and yeast were found to be the most suitable. A num-

ber of substances with anti-inflammatory properties were comparatively tested against these substances. The method for quantitative evaluation of the experiments has been discussed.

Zusammenfassung

An der Pfote der Maus wurden verschiedene Stoffe auf ihre entzündungsauslösende Fähigkeit untersucht. Am besten geeignet für ein Pfotenoedem bei der Maus erwiesen sich Serotonin, Formalin, Silbernitrat und eine Hefesuspension. Eine Anzahl bekannter Verbindungen mit antiphlogistischer Eigenschaft zeigte in vergleichenden Untersuchungen eine deutliche Hemmung der genannten Pfotenoedeme. Der Wert dieser Methode für die quantitative Auswertung antiphlogistisch wirksamer Verbindungen wird diskutiert.

Résumé

Différentes substances provoquant des inflammations, ont été testées sur la patte de la souris. La Sérotonine, le formol, le nitrate d'argent et la levure, ont été trouvés comme les plus pratiques.

Un certain nombre d'autres substances anti-inflammatoires ont été testées comparativement contre ces substances à activité phlogistique.

L'auteur discute la méthode d'évaluation quantitative de ces expériences.

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