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CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT

SOME FACTORS INFLUENCING THE PERCUTANEOUS TOXICITY OF GB

By

M. AINSWORTH

PORTON TECHNICAL PAPER No. 426

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SOME FACTORS INFLUENCING THE PERCUTANEOUS TOXICITY OF GB.

by

M. Ainsworth

INTRODUCTION

GB applied to the skin of animals produces toxic effects which are somewhat unpredictable in degree, and determinations of the LD.50 for depilated rabbits in various laboratories have varied considerably. Marzulli and Williams (1) have pointed out that such estimates have ranged from 1.5 - 7.5 mg./kg. Unless the contamination is covered, a large proportion of the applied GB evaporates (1) and, in the case of rabbits, less than 2% of the dose penetrates the skin (2). The ratio of the effective dose to the applied dose may be written as $A/(A + E)$ where A is the rate of skin absorption and E the rate of loss of the GB by evaporation and hydrolysis. If E is reduced to zero, e.g. when the applied GB is covered to prevent evaporation, the toxicity may rise by nearly two orders (2). It is clear, therefore, that factors which modify the rates of evaporation and skin absorption of GB can profoundly influence its percutaneous toxicity. Some of the factors likely to be significant have been examined in the present experiments.

METHODS

The availability of radio-active GB (P32) prepared by Perry etal (4) made it possible to measure directly the quantity of GB absorbed by the skin. Such estimations, using a liquid-type geiger counter however, required the skin to be digested, and intact rabbits could not be used because of the difficulties of estimating the total amount of GB absorbed systemically. Freshly resected skin was therefore used, and this offered the advantage that comparative tests could be made on the same animal.

Depilation was carried out on the day preceding that of the experiments. Fur on the back of the rabbit was closely clipped over a region approximately 10 x 20 cm., and depilation was completed using a barium sulphide paste. Only the regions of skin which were free from abrasions and other blemishes were used. Immediately before the experiment the animal was killed by an intravenous overdose of Nembutal.

The depilated skin was then removed and cut into pieces of about 4 cm. square, each piece being put on a small plate of aluminium (5 x 6 cm.). This was done for subsequent convenience in manipulation and to ensure good thermal conduction between the skin and a constant temperature surface. The latter was the upper surface of a closed copper bath through which water circulated from a Siemens 'ultrathermostat'. Thermocouple measurements of the surface temperatures of depilated skin on otherwise normal rabbits gave values ranging from 32.8 - 35.0°C., the most common value being 33.5°C. To maintain this temperature at the surface of the resected skin required the water-bath temperature to be 41°C., and therefore the temperature gradient through the skin was probably somewhat steeper than in the intact animal.

The water bath on which the skin samples were placed was enclosed by a small wind tunnel, at one end of which was a speed-controlled fan. The velocity of air moving over the skin surface was measured with a small rotating vane air-speed meter.

Doses of GB were applied to the skin as freely falling droplets delivered from a microburette (5).

The following procedure was adopted for estimating the amount of GB absorbed by the skin. The skin and aluminium plate were transferred from the water bath to a funnel, sprayed with a 20% aqueous solution of Teepol and then washed for 5 minutes under a gentle stream of tap-water to remove any surface GB or products of its hydrolysis. The water was collected in a one-litre volumetric flask for subsequent estimation of the skin surface activity. (Fractional estimations of the P32 activity of the washing water indicated that most of the GB etc. was removed within the first ten seconds of washing). The skin sample was then removed from the plate and digested to a clear solution in 10 ml. of hot nitric acid. The solution was made up to 50 ml. with tap-water and 10 ml. of this counted in an M6 geiger-tube. Counts greater than 5×10^3 were taken, giving estimates reliable (for $p = 0.05$) to $\pm$ 2.8%.

RESULTS

1. Evaporation and absorption of GB from the skin surface.

A number of skin samples from one rabbit were each contaminated with a 3.88 mg. drop of GB. The skin surface temperature was maintained at 33.5°C. and the air-speed over the skin at 250 ft./min. At intervals after the application of the GB skin samples were washed as described, and the amounts of GB remaining on the surface and absorbed into the skin were estimated. The data from two experiments are plotted in Figs. 1 and 2. Fig. 1 shows that the evaporation rate was rapid at first but subsequently became slower. Even after 30 minutes there was a residue on the skin surface, and it seems likely that this was a relatively involatile product of hydrolysis of the GB (3). The decrease in evaporation rate was probably due to the increasing amount of this product.

The absorption curves in Fig. 2 appear to indicate a slow initial rate of absorption of the GB by the skin, but it is possible that the penetration was not deep at this time and that irrigation with a wetting agent may have removed some GB from the stratum corneum in addition to the true surface GB.

The maximum rates of evaporation (Fig. 1) were approximately 5.0 and 4.0 mg./min. and the corresponding skin absorption rates (Fig. 2) were 25 and 40 µg./min. It would seem that for these rabbits the ratios of effective to applied doses would have been approximately 25/5000 and 40/4000 or 1/200 and 1/100.

The skin absorption rate of GB was further investigated in experiments in which the applied GB was immediately covered with nylon sheet to prevent evaporation, the procedure and conditions being essentially the same as in the preceding experiment. Data obtained with three rabbits are given below (Table 1), and values determined using a free-graft sample of human skin are included for comparison.

Table 1

The rate of absorption of liquid GB by skin

Time after application of GB. (min.)	Amounts of GB absorbed (mg.)			
	Rabbit 1	Rabbit 2	Rabbit 3	Human
2	0.16	0.38	-	-
4	0.46	0.47	0.40	0.20
8	1.02	0.90	0.78	0.27
16	2.11	1.73	2.10	0.33
32	3.24	2.17	2.40	0.54
64	3.40	2.44	3.46	0.64

These data are plotted in Fig. 3 and appear to indicate that with no complications due to evaporation, hydrolysis, or spreading of the applied GB (all the doses having spread to the same extent) the absorption rates of GB by the skins of the three rabbits were much the same. By comparison, the absorption rate for human skin was slow, being approximately 1/6 that for rabbit skin.

2. Variation in the rate of absorption of GB with skin temperature.

In general, an elevated temperature increases the rapidity of effect of a drug, the mobility of the drug along the particular route of administration and its rate of reaction with tissues being increased(6) The degree of the effect may be such that the rate of action of the drug is increased by a factor of 2-3 for a temperature rise of 10°C. (7). The temperature of skin can vary over a fairly wide range, depending on the ambient conditions of temperature, humidity, and air speed. The effect of temperature variation on the rate of absorption of GB by skin was therefore examined.

The depilated skins of four rabbits were used, the water-bath temperature being adjusted to give a range of skin surface temperatures in the four groups of samples. These temperatures were measured using copper-constantan thermocouples. Each sample of skin was contaminated with a 3.88 mg. drop of GB, the application being covered immediately with nylon sheet. After various intervals the samples were washed, digested and the absorbed GB estimated. Table 2 gives the results of the experiment.

Table 2

The effect of skin Temperature on the rate of absorption of GB.

Time after application of GB.(min)	Amount of GB absorbed (mg.)			
	Rabbit A. Temp.20°C	Rabbit B. Temp.25°C	Rabbit C. Temp.33.5°C	Rabbit D. Temp.37.5°C
2	0.13	0.12	0.20	0.41
4	0.20	0.31	0.55	0.88
8	0.35	0.56	0.96	1.87
16	0.79	0.92	1.95	2.86

These data clearly show that GB was absorbed by the skin more rapidly at an elevated temperature. The effect of temperature change also appeared to be more significant at the higher temperatures.

A rise in skin temperature also increases the rate of evaporation of the applied GB and this tends to compensate somewhat for the increased skin absorption rate.

The previous experiment was therefore repeated but without covering the GB contamination. The air-speed over the skin was 230 ft./min. The following data were obtained (Table 3), values for GB absorbed being expressed as percentages of the dose applied.

Table 3.

The effect of skin temperature on the rate of absorption of GB.

Rabbit	Skin Surface Temp.°C.	Percentage of applied dose absorbed		
	Time:-	1 min.	5 min.	10 min.
E	20	0.07	0.13	0.70
	27	0.09	0.36	1.20
	34	0.11	0.52	1.01
	37	0.38	0.79	0.92
F	20	0.12	0.16	0.81
	26	0.09	0.34	1.02
	31	0.26	0.31	0.97
	38	0.76	0.66	0.93

Although the amounts of GB absorbed at 1 minute and 5 minutes varied with the skin temperature, those at 10 minutes were much the same, indicating that the total amount of GB absorbed by the skin was independent of temperature.

A further experiment was carried out to check this point. Skin absorptions were measured 10 minutes after applying the GB, the previous data having indicated that, at a skin temperature higher than 20°C., the GB had evaporated and absorption was complete at this time. Depilated skins from three rabbits were used and three different doses were applied. The effect of drop size will be discussed later. The air-speed over the skin was 230 ft./min. The data obtained are given in Table 4 below.

Table 4.

The effect of skin temperature on the total absorption of GB from a freely evaporating contamination

Surface Temp. of skin(°C.)	Percentage of dose absorbed (at 10 min.)			
	Rabbit G (0.2 mg.)	Rabbit H (0.8 mg.)	Rabbit J (3.2 mg.)	Mean
20	0.82	0.70	0.64	0.72
26	1.25	0.58	0.83	0.87
29	0.55	0.88	0.73	0.72
33	0.82	1.22	1.03	1.02
38	0.67	0.78	0.61	0.67
Mean	0.82	0.83	0.77	

It seems clear from these data that the proportion absorbed of the dose of GB applied to the skin is inappreciably affected by skin temperature over the particular range studied. This is doubtlessly due to a similar effect of temperature on the evaporation and skin absorption rates of the GB. Without the compensating mechanism of evaporation, the percutaneous toxicity of GB would depend on the skin temperature. Oberst et al (8) have reported a temperature effect on the percutaneous toxicity of GB vapour when the dose was measured as Ct.

3. The effect of air velocity.

Resected skin samples from a depilated rabbit were exposed to various air speeds and each was contaminated with 1.0 mg. of GB. The skin surface temperature was 34.5°C. at zero air-flow, the effect of increasing the air-speed being only a slight fall to 33.5°C. at 200 ft./min. Ten minutes after applying the GB the skin was washed as already described and the amount of GB absorbed was estimated. The results of three experiments are given below in Table 5.

Table 5

The effect of air-speed on the total skin absorption of GB from a given contamination

Air-speed (ft./min.)	Percentage of dose absorbed		
	Rabbit K.	Rabbit L.	Rabbit M.
0	1.89	3.60	1.50
90	0.88	2.20	0.95
175	0.73	0.90	0.62
280	0.60	-	-
450	0.63	1.20	0.37

Similar experiments were carried out to investigate the effect of varying the air-speed when the skin was covered by clothing. Depilated skin samples from one rabbit were used. In one group, each piece of skin was covered by a piece of battledress serge, and in the other group by an inner layer of angola shirting and an outer layer of the serge. The contamination was 1.0 mg. of GB, and the skin surface temperature 33.5°C. Estimations of the GB absorbed by the skin were made 10 min. after applying the GB. The data from two experiments are presented in Table 6.

Table 6

The effect of air speed on the absorption of GB by clothed skin.

Air-speed (ft./min.)	Percentage of dose absorbed by the skin			
	One layer (Serge)		Two layers (serge + Angola)	
	Rabbit N	Rabbit O	Rabbit N	Rabbit O
0	2.8	1.56	4.2	2.31
95	1.5	0.93	2.0	1.25
172	0.70	0.52	1.4	0.80
500	0.41	0.42	0.62	0.57

The combined data from Tables 5 and 6 are plotted in Fig. 4. For the purpose of comparison, the skin absorption values have been converted into percentages of the absorption at zero air-speed for each group of data. It is seen that all the data can be fairly represented by a single curve, indicating that the effect of a variation in air-speed on the amount of GB absorbed by the skin from a given contamination is the same for bare and for clothed skin.

It is of some interest from the toxicological aspect that this effect on absorption is much reduced when the air-speed is greater than 200 ft./min. (approximately 2 m.p.h.). Air velocities in a fume-cupboard of a type commonly used in toxicological work were studied. The shutter of the cupboard was raised through various distances and the air velocities over the back of a rabbit in the cupboard were measured. These values are plotted in Fig. 5 with the height of the shutter i.e. the distance of opening of the cupboard. In Fig.6 corresponding values from the curves in Figures 4 and 5 are plotted to give a relation between the relative amounts of GB absorbed by the skin and the distance of opening of the fume cupboard. It is clear that in this particular cupboard the percutaneous toxicity of GB to rabbits could vary over a four-fold range depending on the degree to which the cupboard was opened i.e. ventilated during the tests.

4. The effect of Drop Size.

If a drop assumes a penticular shape when it falls on the skin and if ϕ = angle of contact,

V = volume of dose,

n = number of drops,

R = radius of curvature of liquid surface

then it can be shown that:-

$$\frac{V}{n} = \frac{\pi R^3}{3} (2 - 3 \cos \phi + \cos^3 \phi) \qquad \ldots\ldots (1)$$

The area (Ae) of the evaporating surface for a single drop is given by

$$Ae = 2\pi R^2 (1 - \cos \phi) \qquad \ldots\ldots (2)$$

From (1) and (2),

$$Ae = (V/Cn)^{2/3} \qquad \ldots\ldots (3)$$

where C depends on ϕ only (i.e. constant).

The total rate of evaporation (E) is proportional to nAe, i.e.

$$E = K(V/C)^{2/3}\, n^{1/3} \qquad \ldots\ldots (4)$$

where K is a constant of proportionality for a fixed set of conditions. If Ac is the area of liquid in contact with the skin for each drop, it can be shown that:-

$$Ac/Ae = \tfrac{1}{2}(1 + \cos \phi) = \text{a constant} \qquad \ldots\ldots (5)$$

so that if U is the rate of uptake of liquid by the skin, i.e. $U \propto nAc$, then

$$U \propto E \propto n^{1/3} \qquad \ldots\ldots (6)$$

This result indicates that the amount of a liquid, in this case GB, absorbed by the skin from a given contamination is independent of the size of the drops applied.

The effect was examined experimentally. GB in various doses, each as a single drop, was applied to a series of depilated rabbit skin samples. The skin surface temperature and the air-speed were respectively 33.5°C. and 250 ft./min. By the procedure already described, the amounts of GB absorbed by the skin samples 10 minutes after the applications were estimated. These values of skin absorption expressed as the percentages of the doses applied are given in Table 7.

In the same experiment the effect of variation in drop size was examined for clothed skin. In this case the preceding theoretical consideration does not apply, for GB spreads rapidly in cloth and experiments, which will be reported elsewhere, have shown that with most clothing materials no liquid GB penetrates to the surface of the skin. The skin absorbs GB vapour from the surface contamination. The experimental procedure was essentially the same as that for the unclothed skin except that in one group the skin samples were covered with one layer of serge and in another group with an inner layer of angola and an outer layer of serge. For comparison purposes, applications of the GB were made to unclothed and clothed skin at the same time and using skins samples from the same animal. In Table 7 the amounts of GB absorbed as percentages of the applied doses are expressed as the means of several estimations together with the standard deviations.

Table 7

The effect of Drop Size on the Absorption of GB by unclothed and clothed skin.

Dose applied as one drop (mg.)	No. of estimates	Percentage of Dose absorbed		
		Unclothed Skin	Clothed Skin (Serge)	Clothed Skin (Angola + Serge)
0.20	8	1.21 ± 0.21	0.97 ± 0.25	1.16 ± 0.31
0.50	9	0.97 ± 0.18	0.91 ± 0.22	1.09 ± 0.19
1.00	8	1.07 ± 0.33	1.00 ± 0.31	1.21 ± 0.36
4.00	8	0.93 ± 0.27	0.78 ± 0.24	0.91 ± 0.28

In the three groups of data there appear to be no significant trends with drop size in the proportion absorbed of the applied dose of GB.

Drops of GB on depilated rabbit skin have in many cases been observed to remain discrete for some time. In some cases, however, and especially on skin which is incompletely depilated or pigmented, the drop immediately spreads. This behaviour would be expected slightly to increase the absorbed proportion of the GB by making the ratio of skin contact area to evaporating area equal to unity. For a drop remaining lenticular in shape this ratio is less than unity (equation 6). Spreading of the drop, by increasing the area of contact of the GB and the skin, would also appreciably increase the rate of systemic absorption of the GB. Such unpredictable behaviour of the drop on the skin might be a factor contributing to the individual variation in susceptibility so well known in percutaneous toxicological experiments.

SUMMARY

1. Drops of GB were found to evaporate within two minutes from depilated rabbit skin maintained at a surface temperature of 33.5°C. and in an air-stream of 250 ft./min. The proportion of the drop absorbed by the skin was approximately 1%.

2. When the contamination was immediately covered to prevent evaporation, the skin continued to absorb GB at a steady rate. This rate for a sample of human skin was approximately one sixth of that for depilated rabbit skin under the same conditions.

3. Skin surface temperature affected the rate of GB absorption, an increase in temperature increasing the rate. Changes in evaporation rate of the GB appeared to compensate for this effect so that the amount of GB absorbed from a given skin contamination was unaffected by skin temperature.

4. Variation in air-speed over the skin appreciably modified the proportion of GB absorbed when the skin was uncovered or covered with one or two layers of cloth. In the range of air velocity 0-200 ft./min. (0 - 2 m.p.h.) the amount of GB absorbed by rabbit skin from a given contamination varied over a three-fold range. The effect could be minimised in percutaneous experiments with GB, by maintaining an air-velocity over the skin in excess of 200 ft./min.

5. The fraction of GB absorbed by the skin (unclothed or clothed) was independent of the size of GB drop applied.

MA/PMF.

(Sgd.) H. Cullumbine,
Supt., Medical Division.

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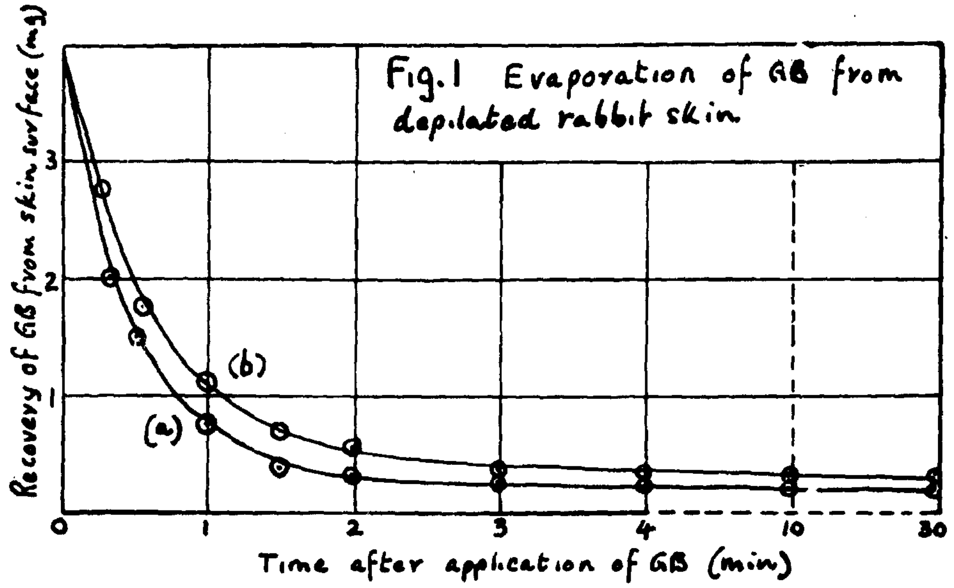
Fig. 1 Evaporation of GB from depilated rabbit skin
(a)
(b)
Recovery of GB from skin surface (mg)
Time after application of GB (min)
0
1
2
3
4
10
30

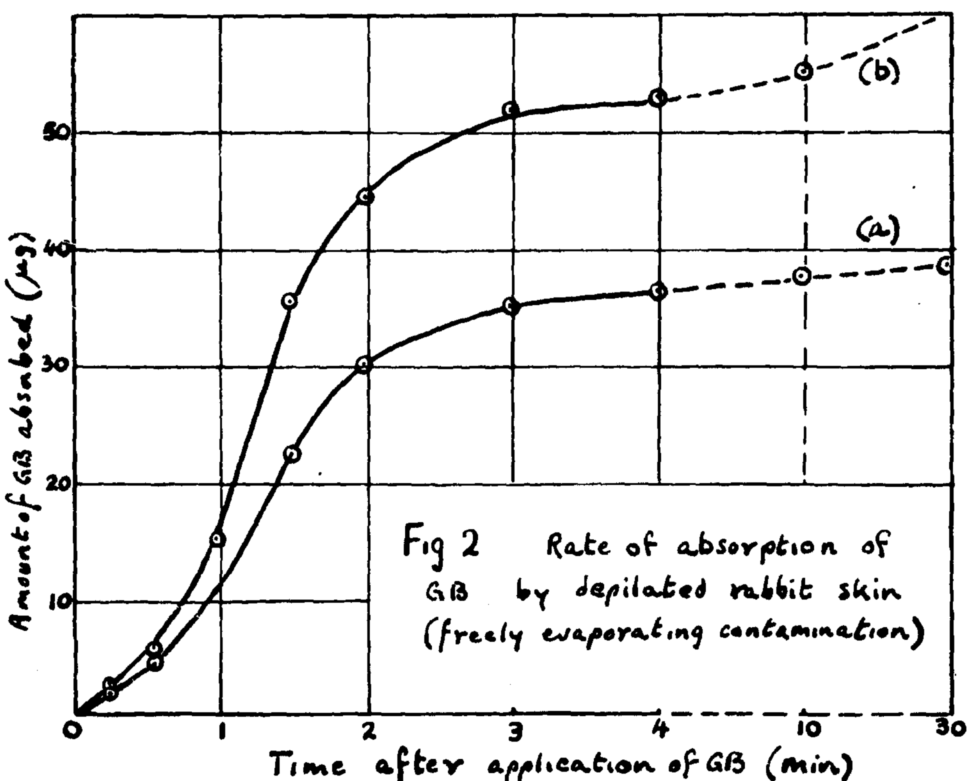
Fig 2 Rate of absorption of GB by depilated rabbit skin (freely evaporating contamination)
(a)
(b)
Amount of GB absorbed (μg)
10
20
30
40
50
Time after application of GB (min)
0
1
2
3
4
10
30

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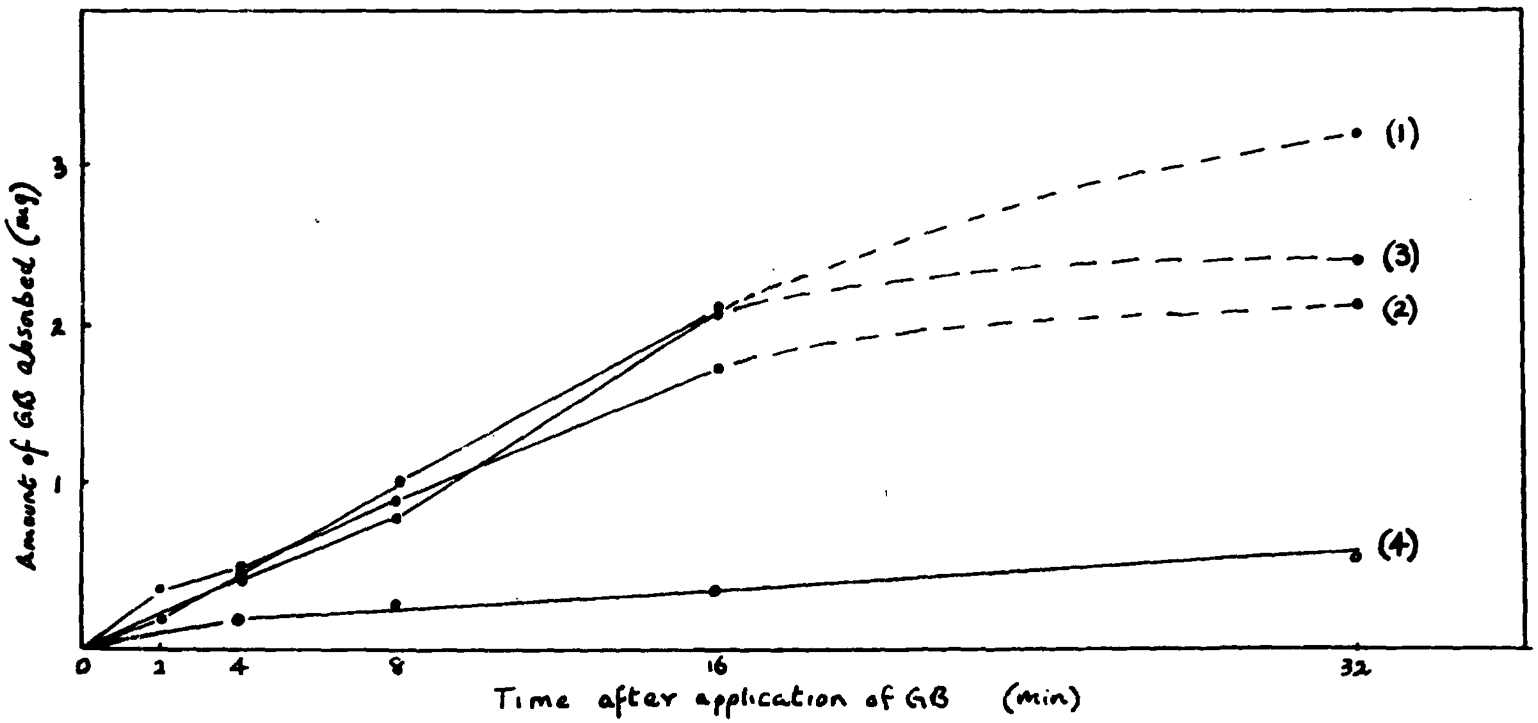

Fig 3 Rates of absorption of GB by skin (1-3 rabbits, 4 human)

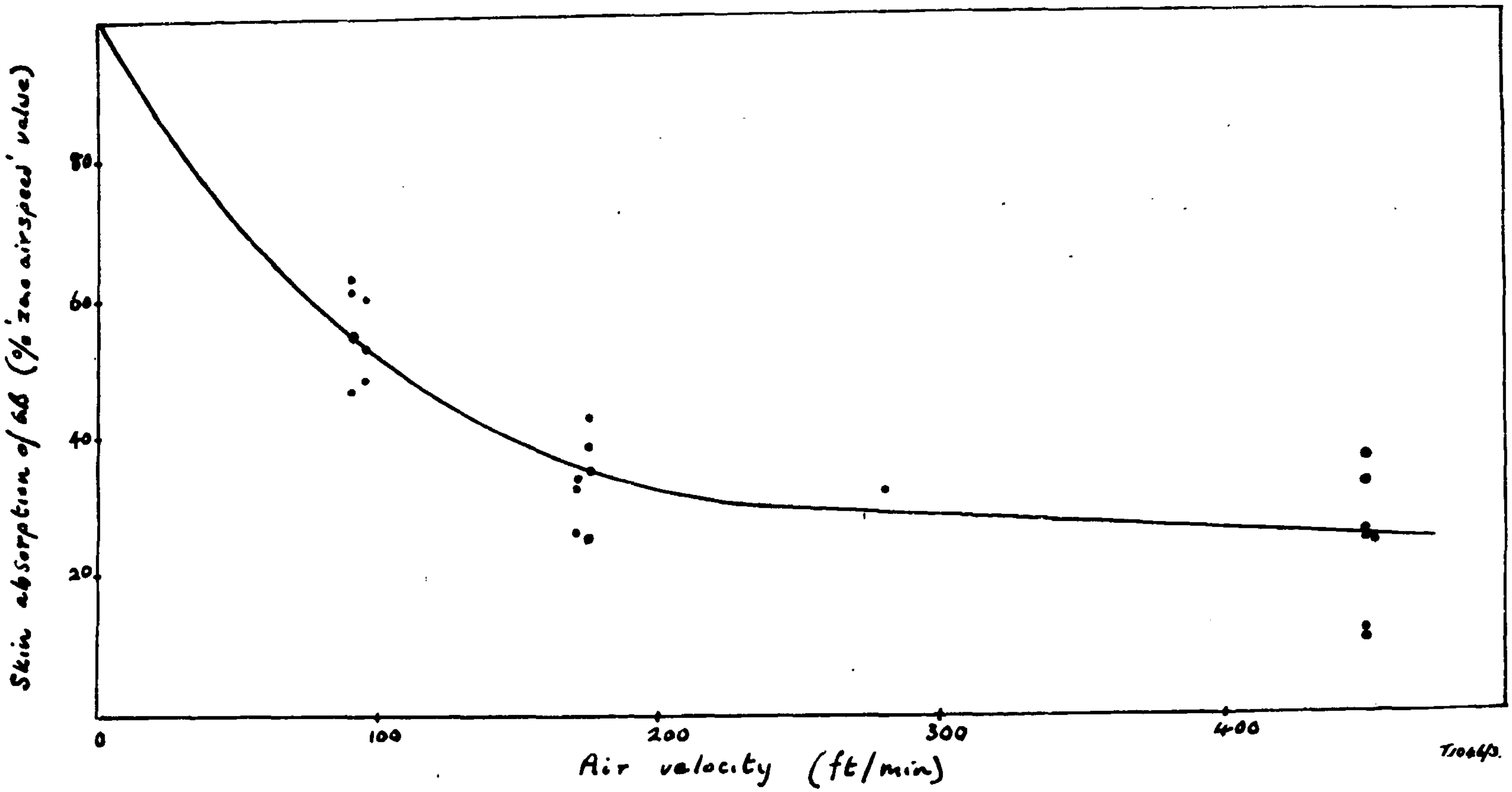

Fig.4 The effect of air velocity on the absorption of GB by skin

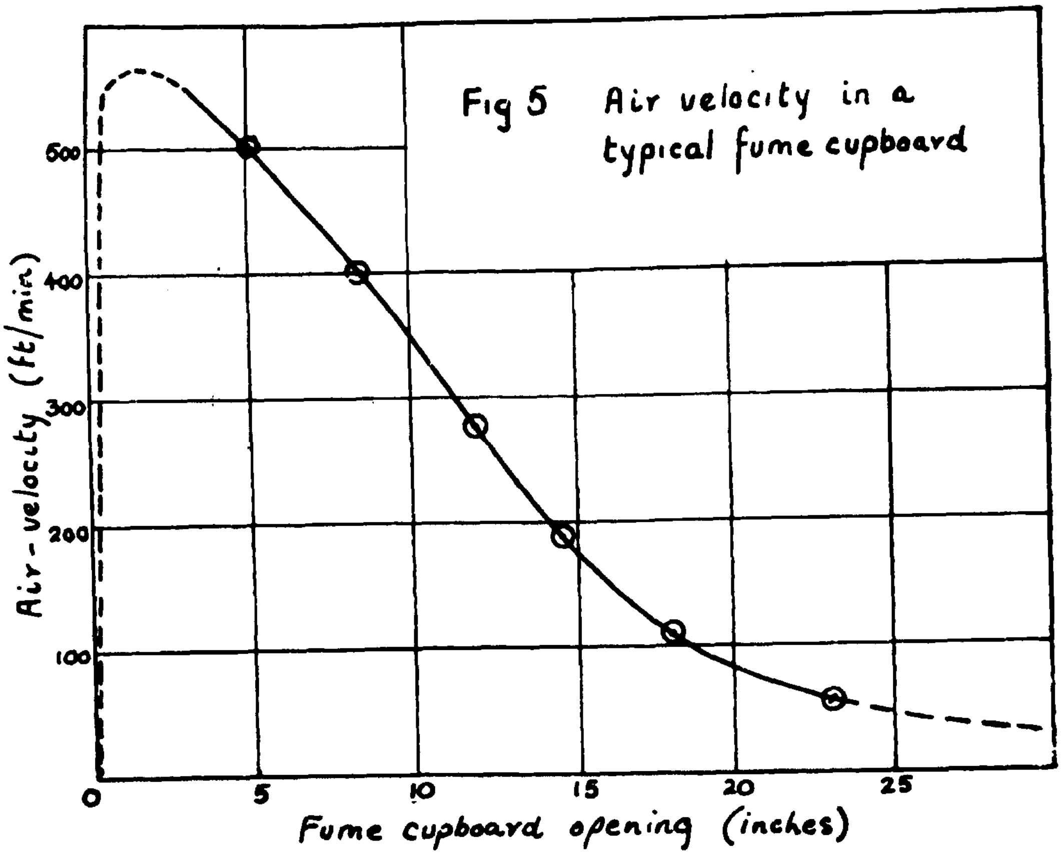
Fig 5 Air velocity in a typical fume cupboard
Air-velocity (ft/min)
500
400
300
200
100
0
5
10
15
20
25
Fume cupboard opening (inches)

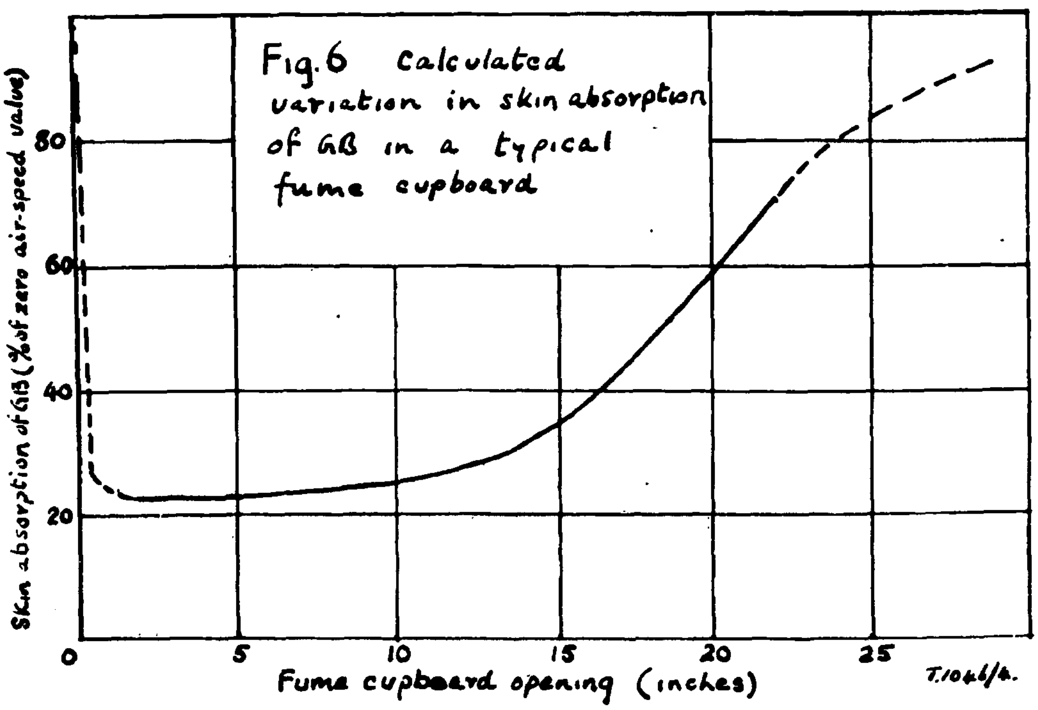
Fig. 6 Calculated variation in skin absorption of GB in a typical fume cupboard
Skin absorption of GB (% of zero air-speed value)
80
60
40
20
0
5
10
15
20
25
Fume cupboard opening (inches)

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