PERCUTANEOUS ABSORPTION USING DIIODOFLUORESCEIN ^{131}I

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Many different methods have been described for studying the absorption of medicaments from ointment bases applied to the intact skin. Some have been based on the application of pharmacologically active substances such as alkaloids[1] and anticholinesterase drugs. We have previously described a method using the anticholinesterase drug eserine in the rat[2]. Other methods have been based on the inunction of an easily recognised substance such as potassium iodide or a salicylate[3–6] which can be estimated chemically in the blood, urine or faeces.

The introduction of radioactive tracer substances, which can be readily detected in biological materials, even in minute quantities, offers a new approach with the advantage of greater sensitivity[7–9] We have made a preliminary study of the percutaneous absorption of diiodofluorescein ^{131}I in the rat from a number of typical ointment bases with two main objects in view: (*a*) to estimate the value of radioactive tracer techniques in assessing percutaneous absorption in laboratory animals and (*b*) to obtain information on the absorption of medicaments from white soft paraffin, lard, cetomacrogol, hydrous ointment and hydrous emulsifying ointment.

Experimental

Materials

Diiodofluorescein ^{131}I was chosen because it was readily available and possessed solubility characters similar to those of a number of substances commonly applied to the skin. It is only sparingly soluble in water and slightly soluble in chloroform, ether and fixed oils. But it is readily soluble in ethanol and aqueous solutions of propylene glycol. Its hydrophilic and lipophilic properties are therefore fairly evenly balanced. ^{131}I has the advantage of a short half life, eight days, which is a long enough time for the experiments to be performed but does not require a long storage for decontamination of apparatus and animals.

Methods

The preparation of ointments containing radioactive diiodofluorescein raised some interesting pharmaceutical problems.

Preparation of Ointments

White Soft Paraffin and Lard. Non-emulsified ointments, in which the diiodofluorescein was insoluble, were prepared by the following technique:

Diiodofluorescein ^{131}I, approximately	20 mg.*
Base	5 g.

The base was melted in an evaporating dish over a water bath and the diiodofluorescein transferred to a warm glass mortar. The medicament was triturated with the melted base and the ointment transferred to a modified "Plim" syringe, described below. In carrying out these operations, the hands were protected by rubber gloves to prevent skin contamination and a lead screen (thickness 1½ inch) was interposed between the operator and the radioactive material to minimise exposure to radiation.

Cetomacrogol Base. Non-emulsified ointments in which the diiodofluorescein was completely soluble were prepared as follows:

Diiodofluorescein ^{131}I, approximately	20 mg.*
Propylene Glycol	3 g.
Cetomacrogol	2 g.

The cetomacrogol was dissolved in the propylene glycol by heating over a water bath. The diiodofluorescein was added and the ointment heated until solution was complete. It was then transferred to the modified "Plim" syringe.

Hydrous Ointment. Emulsified ointments were prepared as follows:

Diiodofluorescein ^{131}I, approximately	20 mg.*
Propylene Glycol	1·25 g.
Purified Water	1·25 g.
Ointment of Wool Alcohols	2·5 g.

The ointment of wool alcohols was melted in an evaporating dish over a water bath. The diiodofluorescein was dissolved in a mixture of the propylene glycol and purified water and the solution, after heating over a water bath, was transferred to the melted base. The mixture was stirred until cool and transferred to the modified "Plim" syringe.

Hydrous Emulsifying Ointment.

Diiodofluorescein ^{131}I, approximately	20 mg.*
Propylene Glycol	1·25 g.
Purified Water	1·25 g.
Emulsifying Ointment	2·5 g.

This ointment was prepared by a similar technique.

Measurement of Dose. In measuring the amounts of ointment to be applied to the skin, a method was devised to avoid excessive manipulation of the ointment. The ointment was placed in a special syringe so that it could be subdivided into doses without weighing. This syringe is illustrated in Figure 1. It consists of a "Plim" syringe in which the piston has been replaced by one actuated by a screw-thread. The nozzle of the syringe can be removed for filling the barrel with ointment. When

* The actual amount of diiodofluorescein varied with different batches of material. The quantity used was estimated to give a total activity of approximately 2 mc. in 5 g. of ointment.

the screw-thread is turned, the ointment is extruded in an amount determined by the number of turns of the thread. The activity of each dose was determined as described under physical methods.

Animal Experiments. Twelve rats of the same sex, weighing about 180 g., were used in each experiment. On the day before the experiment, the hair was removed from the skin of the back with electric clippers and any rats which showed cutaneous lesions were rejected. To prevent oral contamination and to facilitate handling, the rats were anæsthetised with an intraperitoneal injection of urethane (0·4 ml. of a 25 per cent. solution per 100 g. body weight). In addition, a cardboard collar was fixed round the chest as an added safeguard. Each rat was injected subcutaneously with heparin (200 units per 100 g. body weight) to prevent clotting during collection of the blood. The measured amount of ointment was applied with a metal spatula to an area of 4 cm. square marked on the back, and rubbed in with the spatula for exactly one minute. The rats were then placed into individual tins lined with cartridge paper. In each experiment, three rats were killed after one, two, three and four hours, and blood was collected from the heart by the following procedure to avoid contamination. The rat was deeply anæsthetised with chloroform and then placed on its back on a piece of cotton wool in a lined enamelled tray. The skin was removed from the thorax and abdomen, using instruments reserved for the purpose, since the fur usually became contaminated with radioactive material. The thorax was then opened with new instruments, by cutting through the sternum, and blood was removed from the heart with a teat pipette and measured in a 10 ml. measuring cylinder containing heparin. The volume of each blood sample was recorded and it was then transferred to a labelled test-tube for subsequent counting.

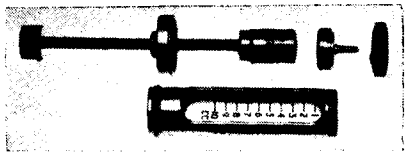

FIG. 1. Modified "Plim" syringe.

Physical Methods. The ointment was divided into 12 approximately equal amounts and the relative activities accurately compared by measuring each at 25 cm. from a Geiger Muller tube (G10 Pb cathode). The amount of blood obtained from the rats varied but, for measuring purposes, it was diluted to 10 ml. and counted using an Ekco scintillation counter. From previous work with ^{131}I, the ratio of the counting rate on an Ekco scintillation counter, from a source diluted to 1 litre, to the counting rate due to the same source at 25 cm. from the G.M. was known to be 43·4. It was therefore possible to relate the radioactivity of the blood to the amount of ointment applied and to determine the proportion absorbed. Since the blood volumes of the rats were unknown, the results have been expressed as percentage of diiodofluorescein absorbed per 10 ml. of blood.

If D = counting rate of ointment at 25 cm. from G.M. tube
A = „ „ „ 10 ml. of blood

Then, ointment diluted to 1000 ml. would give a counting rate of $43{\cdot}4 \times D$ in scintillation counter, and the percentage absorbed into 10 ml. of blood $= \frac{A}{43{\cdot}4D}$

RESULTS

The absorption obtained in different animals showed considerable variation. This is illustrated in Table I, which gives the results obtained with the cetomacrogol/propylene glycol base. The results obtained with

TABLE I

ABSORPTION OF DIIODOFLUORESCEIN FROM CETOMACROGOL BASE

Time (min.)	Dose*	Blood activity†	Absorbed‡ per cent.	Average
60	2667	31	0·0003	0·0002
60	2544	12	0·0001	
60	2591	15	0·0001	
120	2684	14	0·0001	0·0005
120	2740	96	0·0008	
120	2676	74	0·0006	
180	2579	68	0·0006	0·0019
180	2542	—	0·0045	
180	2597	62	0·0005	
240	2792	79	0·0006	0·0035
240	2696	948	0·0081	
240	2628	210	0·0018	

* Counts per minute determined at 25 cm. distance from Geiger Muller tube (G10 Pb cathode) = D.
† Counts per minute of 10 ml. blood determined in Ekco scintillation counter = A.
‡ Per cent. absorbed per 10 ml. of blood = $\frac{A}{43{\cdot}4D}$.

the five different bases are shown in Table II and illustrated in Figure 2. They show that diiodofluorescein was absorbed from all the ointments we tested and that the amount absorbed increased with increasing time, with the possible exception of white soft paraffin. Compared with the initial doses, the amount absorbed was extremely small. Because of the

TABLE II

ABSORPTION OF DIIODOFLUORESCEIN FROM DIFFERENT OINTMENT BASES

Time (min.)	Per cent. of applied dose absorbed per 10 ml. blood = A/43·4D				
	Soft paraffin	Lard	Cetomacrogol	Hydrous ointment	Hydrous emuls. ointment
60	0·0004	0·0004	0·0002	0·0003	0·0003
120	0·0008	0·0006	0·0005	0·0009	0·0004
180	0·0010	0·0007	0·0019	0·0008	0·0007
240	0·0005	0·0023	0·0035	0·0030	0·0008

wide animal variations, we have not attempted to evaluate our results statistically. To obtain results suitable for statistical analysis, large numbers of rats would have to be used, raising difficulties of expense, radiation hazards and disposal of contaminated animals. Our results were most variable with the bases, lard and white soft paraffin, in which the diiodofluorescein was insoluble. Absorption was best from cetomacrogol and hydrous ointment, in both of which the diiodofluorescein

was dissolved by adding propylene glycol. With the cetomacrogol base, the tracer was dissolved in a homogeneous mixture, whereas in the hydrous ointment, it was present in the dispersed phase of a water-in-oil emulsion. Absorption was poor from hydrous emulsifying ointment in which the diiodofluorescein was again dissolved in propylene glycol, but here it was present in the continuous phase of an oil-in-water emulsion.

A possible explanation of these findings can be made on the theory that the skin surface is repellent to aqueous solutions, and, in the case of oil-in-water bases, the external aqueous phase has a retarding effect on absorption. When the external phase is oily, as in hydrous ointment, absorption is facilitated by miscibility with the sebum, which allows the medicament to come into contact with the absorbing cells at the base of the follicles. Cetomacrogol, possessing both lipophilic and hydrophilic properties, may therefore assist penetration and absorption by a similar mechanism.

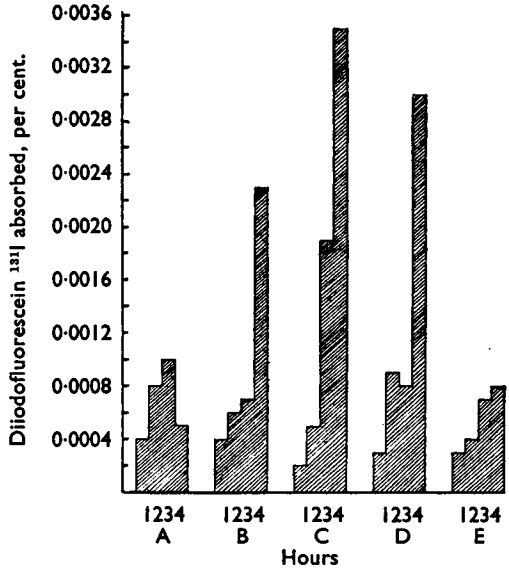

FIG. 2. Absorption of diiodofluorescein ^{131}I from five different ointment bases. A. Soft paraffin. B. Lard. C. Cetomacrogol. D. Hydrous ointment. E. Hydrous emulsifying ointment.

SUMMARY

1. The percutaneous absorption of diiodofluorescein ^{131}I from five different ointment bases in rats was tested.

2. The amount absorbed was extremely small and there was a wide variation between individuals. The numbers needed to obtain an accurate answer would considerably increase the radiation hazards.

3. Absorption was better from hydrous ointment and cetomacrogol than from lard, white soft paraffin and hydrous emulsifying ointment.

The authors wish to thank Ann Coram, Marion Hodges and Pamela Middleton for their assistance in carrying out the experimental work. They also wish to express their thanks to Mr. J. Shephard for supplying the photograph.

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DISCUSSION

The paper was presented by MR. J. W. HADGRAFT.

MR. J. H. OAKLEY (London) said it was disappointing that the newer hydrous emulsifying ointment base had given such poor results. It would be interesting to see the result of using emulsifying ointment as a base instead of hydrous emulsifying ointment, because the lyophobic nature of the base allowed penetration of the skin; the ointment could be easily emulsified and removed from the skin when required.

MR. S. G. E. STEVENS (London) said that a recent paper in the *Journal of the American Pharmaceutical Association* had drawn attention to the increase in penetration of ointment bases incorporating hyaluronidase. The advantage of hyaluronidase seemed to be that non-aqueous ointment bases could be used with very high absorption.

MR. R. L. STEPHENS (Brighton) asked for an assurance that the figures in Table II were the averages for three animals.

DR. K. R. CAPPER (London) suggested that it was possible, even with a small number of animals, to assess statistically the significance of the results, and he doubted whether there was any significant difference between lard, cetomacrogol and hydrous ointment.

DR. B. A. MULLEY (London) asked if the authors could suggest the reason for the fact that in Figure 2, experiments B and D, there was a sudden increase in the absorption of diiodofluorescein between hours 3 and 4. It appeared from the results that the rate of absorption was still increasing in certain experiments. Had the authors made any measurements over longer periods of time?

MR. N. J. VAN ABBÉ (Loughborough) asked the authors to comment on the validity of drawing any conclusions about human percutaneous absorption from experiments with rats. The conclusions in relation to oil-in-water emulsions would be affected by the time of inunction during the experiment, and by the thickness of the film applied. In many oil-in-water emulsions there would be loss of continuous phase, and there might even be reversal of the emulsion after four hours. Percutaneous absorption would depend largely on the nature of the emulsifying agent.

MR. K. L. SMITH (Nottingham) said that one ought to be careful of the conclusions drawn unless they were shown to be statistically significant.

MR. D. H. O. GEMMELL (Glasgow) asked if any attempt was made to estimate the iodine present in the urine of the rats. Was not the concentration of the ointment more important than the amount applied.

DR. G. BROWNLEE (London) said it was intriguing why more diiodofluorescein had not passed from the skin into the animal. By measuring

the concentration of radioactivity in the blood, the assumption was made that there was a relation between blood radioactivity and the diiodofluorescein which had passed through the skin. The authors might comment on whether there was any dye in the urine or staining fatty tissue.

MR. J. B. LLOYD (Manchester) asked if the authors had considered the possible effect of characteristics such as molecular size, basicity and acidity on absorption.

MR. W. P. HUTCHINSON (Oxford) said that by tracer technique in the case of iodine the minimum weight which could be detected using a Geiger counter was 10^{-12} g. It was necessary, therefore, to mix the isotopes carefully into the ointment base. The accuracy of the counter was of the order of about 10 per cent. and it was necessary to do 12 counts and then take the mean.

MR. T. D. WHITTET asked whether the authors had considered measuring the rate of decay of radioactivity at the site of application as a measure of the absorption.

MR. J. W. HADGRAFT, in reply, said that it did not necessarily indicate disappointing results if the amount of systemic absorption from an ointment base was poor. In general one used ointments for localised action, and in many cases systemic absorption was not wanted when an ointment was applied. The authors were concerned not only with determining the systemic absorption from ointment bases, but they hoped at a later stage to examine the levels of penetration of ointments. The incorporation of hyaluronidase had not been considered. The results given in Table II represented an average of a minimum of three animals, and in the case of hydrous emulsifying ointment, soft paraffin and lard, averages of more than three animals. The urine of the animals had not been examined for iodine, but in a number of experiments radioactive iodine was detected in the thyroids. He agreed that diiodofluorescein was not an ideal substance for the study of percutaneous absorption, but the difficulty was to obtain substances in a radioactive state, and diiodoflorescein appeared to be the most satisfactory of those available from the point of view of solubility and radioactivity. The mixing of the tracer in the base was checked in each experiment. If the medicament had not been evenly dispersed through the base it would have shown up in the variation in the dose measured by means of the syringe which was used. The determination of the decay of radioactivity at the site of application had not been considered.

DR. G. F. SOMERS, in reply, said that the conclusions had, perhaps, been drawn a little too strongly, and that it might be more accurate to say that the results "suggested". Referring to the question of increase in rate of absorption, the sudden increase was a reflection of absorption and excretion, both of which must be going on at the same time. The tissues had not been examined, but a great deal of radioactive material was left on the skin. Over 24 hours something different might happen, but it was difficult to maintain the rat under anaesthesia for that period.