Med. exp. 8: 200–208 (1963)

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Preliminary Studies of the Distribution and Fate of ^{82}Br-labelled 2-brom-D-Lysergic Acid Diethylamide (BOL) in the Human Body*

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Although several studies have been made on the distribution and fate of lysergic acid diethylamide in the animal body (1, 2, 3, 4, 5), little is known about it in the human. The very small dosage used in human subjects (1 to 2 γ/kg) makes it nearly impossible to investigate the route of the drug in man with chemical methods. Also, C^{14}-labelled LSD cannot be used for external monitoring studies in man because the low energy β-ray of this isotope does not escape the body.

Further, since there are known to be large variations in the distribution of this compound in different animal species (1), it is to be expected that extension of information from such studies to humans might not be valid. We believed that with 2-brom-D-lysergic acid diethylamide labelled with ^{82}Br it would be possible to study the distribution and fate of this compound in the human body. ^{82}Br emits γ-rays with energies primarily between 0.5 and 0.8 mev, and with its short half-life of 35.9 hours, the total radiation dose to a human subject can be kept quite low. Despite the fact that the bromine derivative D-2-brom-lysergic acid diethylamide (BOL-148) does not show the psychotropic effects of LSD-25, BOL has aroused widespread interest because of its ability to antagonize serotonin and its chemical similarity to LSD-25. Aside from mild sedation, which is the only central effect known, BOL is peripherally a serotonin antagonist presumably even more powerful than LSD-25. It is well known that the psychotomimetic action of LSD-25 is struc-

* This work was supported by the U.S. Atomic Energy Commission.

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Received: March 4th, 1963.

ture-specific, nevertheless one would expect no great differences between the distribution of LSD and brom-LSD in the human body, since it is well established that the passage of drugs across body membranes (6) is determined by the lipid-solubility, ionization, and molecular size of the drug. These factors are very similar both in LSD and BOL. Thus it should be possible to extrapolate data obtained from studies with BOL to elucidate the distribution and fate of LSD-25.

Methods

Subjects used in this experiment were three normal healthy white males, 28, 29 and 34 years old, weighing 166, 177 and 145 lbs. respectively. In each experiment 20 μc of ^{82}bromide-LSD was given intravenously; the amount of BOL-148 was in two cases 40 gamma in 1.7 ml saline, and in one case 800 gamma in 12 ml saline. The ^{82}Br labelled BOL was synthesized in this laboratory; the method will be described in detail elsewhere (10).

The BOL was administered while the subject was lying on the table of a multiple port counter with five scintillation detectors and simultaneous five channel recorder (as built and used for *in vivo* measurements in this laboratory). The *in vivo* surface counting was performed by placing $1\text{-}^3/_4 \times 2$ inch mobile scintillation crystal counters with wide angle collimation over liver and spleen in the midaxillary line and at the posterior mid-line under the upper broad portion of the sacrum (in this experiment the sacrum counter viewed essentially the lower abdomen); lower thigh was used for activity in skeletal muscle. One counter was located at the upper part of the side of the head in order to measure the amount of radioactivity in the brain. The measurements were performed continuously for the first hour after injection and thereafter at intervals of several hours and on the following two days. Data were simultaneously plotted by an automatic recorder.

One hour after injection the subject was placed under a combined optical and γ-ray scanner as described by *H. O. Anger and G. M. Tisljar-Lentulis* (7).

Two hours after injection, and on each following day, the total remaining radioactivity of the subjects was measured in the low background human whole body counter of this Laboratory (8). The urine and feces were collected and also measured in the whole body counter.

Ten milliliter blood samples were taken from the subject at intervals after injection. Hematocrits were determined on all samples. Two milliliter aliquots of plasma and whole blood were counted in an automatic scintillation well counter (Nuclear Chicago). All measurements of activity shown are corrected for background and decay of the 35.9 hour ^{82}Br.

Results

The measurements obtained by the multiple port counter were similar in each of the three subjects. Although it is not valid to

compare exactly the amount of activity in the various organs by comparing the counting rate from the corresponding counters, because the volume of tissue viewed by each counter is not the same, the volumes are sufficiently similar that a qualitative comparison can be made.

Typical curves are shown in Fig. 1; the activity in the spleen and head reached the highest point two to four minutes after injection, while the liver uptake increased over a period of 12 to 14 minutes. Ten minutes after injection the concentration of BOL-148 in the organs was, in the order of decreasing magnitude, as follows: liver, spleen, sacrum, head and thigh (not shown in the figure). One hour later there was a considerable decrease of activity in liver, while the rate of decrease from the head and spleen was slower, and the amount in the sacrum was increasing.

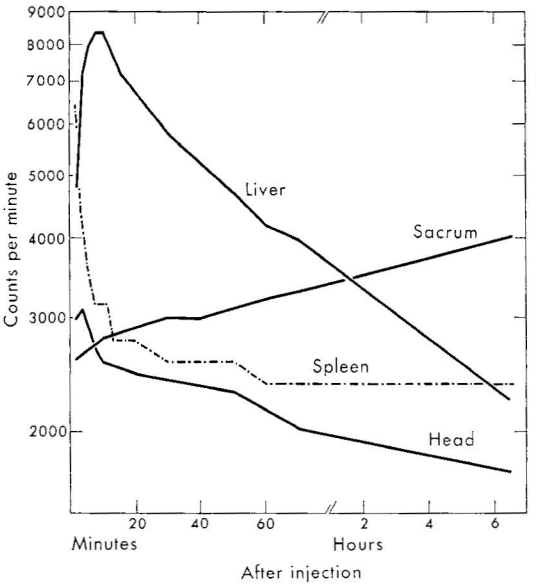

Fig. 1. ^{82}Br activity in various organs measured by multiple port counter.

Continued measurement of the radioactivity in the different organs showed that almost half of the BOL had disappeared from the liver in one hour, while it was increasing in the lower abdomen. Ten hours after injection there was a steady but slow decrease of activity in all organs.

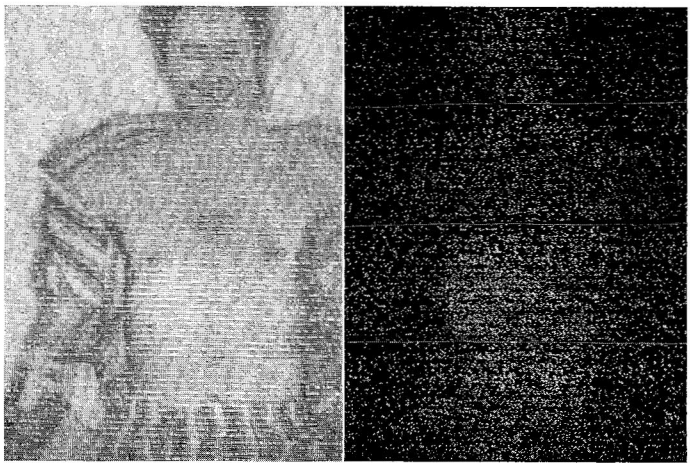

Fig. 2. Localization of ^{82}Br activity at 1.5 hours after injection as measured by the whole body scanner. The two pictures are exactly superimposable, so that localization of activity can be compared to anatomical points on the body.

In Fig. 2 the picture taken with a whole body scanner 1.5 hours after injection shows concentration of the ^{82}Br in the liver, the intestinal tract and possibly bladder, while the amount of tracer in the brain was relatively low. It is to be noted that no activity was seen in the lung.

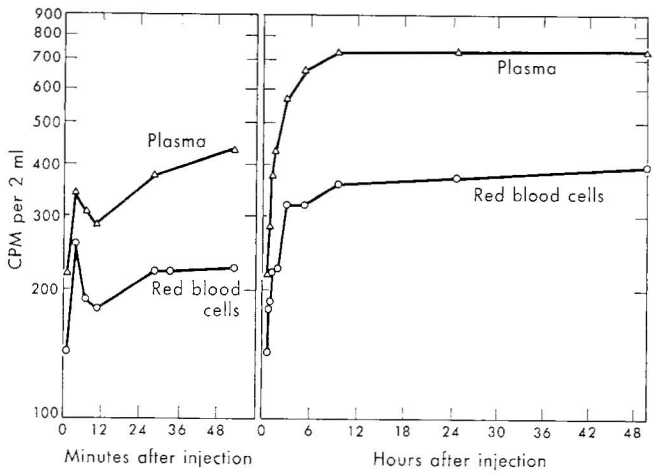

Fig. 3. Activity of ^{82}Br in plasma and in formed elements of the blood ("red blood cells" in figure) as a function of time after injection.

The content of the ^{82}Br-labelled BOL-148 in the plasma and formed elements is shown in Fig. 3. The rise of activity within the first four minutes after injection was followed by a decrease until twelve minutes, after which the activity rose again until it reached its highest level nine hours later. This level was then essentially constant for 48 hours. The formed elements (which included red cells, platelets and white cells) contained about half as much activity as the plasma.

Discussion

These results indicate clearly that there is no single organ which stores BOL for any length of time. It appears that there is an initial rapid diffusion from the injected bolus into tissue which then diffuses back into blood in the first four minutes. The liver then removes the BOL from the blood, and after a period of only 12 to 14 minutes begins to release the ^{82}Br into the blood again. The initially high levels in the other organs represent primarily the high levels in blood before it is cleared by the liver, since blood circulates through these organs. The sacrum count, which as stated previously represents essentially the activity of the lower intestinal tract, reaches a peak at 3 to 6 hours. The activity level in all the organs then declined gradually.

The scanner picture (Fig. 2) clearly shows concentration of activity in the liver and lower intestinal tract, and only slight concentration in the head. The lack of activity in the lungs is in marked contrast to the high levels of LSD found in the lung in mice, rats and monkeys (1, 2, 3). However, the animal data were based on activity per gram of tissue and this is not strictly comparable to external monitoring of activity in lung tissue of average density lower than other tissue. *Doepfner* (3) found that LSD and UML (a derivate of LSD with antiserotonin activity similar to BOL) were distributed almost identically in rats.

Our findings of activity in the blood in humans were not in agreement with those for LSD in animals found by other workers (1, 2). In animals, after the initial rapid clearance from the blood, the level of LSD did not rise again. In our experiments the ^{82}Br activity rose again after 12 to 14 minutes; the chemical form of this activity has not yet been determined. Activity in plasma was found to be approximately twice that in the formed blood elements, but the ^{82}Br levels in each fraction changed with time in about the same way.

Typical whole body counts and measurements of excreta are shown in Table I. It can be seen that the radioactivity is excreted relatively slowly, the whole body count falling to 50% of the injected dose at six days. In the first 24 hours 8.8% of the activity was excreted in the urine, but by the eighth day only 3.3% was lost daily. Especially interesting is the fact that although the activity apparently was initially highly concentrated in the intestinal tract, the average excretion in the stool was only 9% of the urinary excretion.

TABLE I

Days after Injection	Percent of Injected Dose			
	Remaining in Whole Body	Excreted in Urine per Day	Excreted in Feces per Day	Total Excretion per Day
1	90.9	8.4	0.4	8.8
2	76.5	6.2	0.7	6.9
3	66.9	3.8	0.5	4.3
4	61.9	3.7	0.6	4.3
5	54.1	3.2	0.3	3.5
6	50.7	2.9	0.4	3.3
7	46.5	3.9	0.2	4.1
8	45.9	3.1	0.2	3.3

In preliminary biochemical studies of the urine with ion exchange columns, it was found that in the first six hours 17% of the excreted ^{82}Br activity occurred as bromide, while in the 6 to 18 hours after injection the bromide rose to 34%. In addition to this change in relative distribution, the total amount of each fraction was reduced to half the amount excreted per unit time in the first six hours. The remainder of the ^{82}Br was organically bound to as yet unidentified compounds, although it was possible to establish that no ^{82}Br was bound to excreted 5-hydroxy indole acetic acid.

This report is preliminary, and will be reported more fully at a later date with further studies on the effect of cross-tolerance of LSD-25 on Br^{82}-BOL distribution, and on the excreted metabolites. However, it is possible from the data presented here to make the following general summary of the distribution and fate of BOL in the human body:

After intravenous injection, the BOL is taken up by the liver and several hours later is found in the intestinal tract. After 14 to

20 minutes the liver begins to release the ^{82}Br again with a half-time of approximately one hour. Scanner pictures (not shown here) taken at six hours indicate that activity is no longer concentrated in the liver, but is still present in the intestines. There is a small short-lived concentration in the head, and no concentration in the lungs at any time. The concentration in the intestines and the small amount of activity excreted in the stool suggest that BOL is excreted via the bile and reabsorbed in the intestines, as suggested by other workers (1). Since serotonin is known to occur in the intestines and to mediate peristaltic action, the concentration of BOL in the intestine might be related to the anti-serotonin activity of BOL. The spleen, a known storage depot of serotonin in rabbits (9) showed no accumulation of BOL, however.

It is of course apparent that concentration of a compound in an organ does not necessarily mean that that organ is the site of the drug's major pharmacological action. Nevertheless, study of the time course of distribution can yield valuable information about the metabolism of a drug. Because of the evident discrepancies in metabolism and action of psychoactive drugs and their homologs in animals and in humans, it is of great importance in the study of the relationship of such drugs to mental disease to be able to study these compounds *in vivo* in humans. Further experiments along these lines are planned.

Summary

In a study of ^{82}Br-BOL in humans, the time course of distribution was followed in liver, spleen, intestines, lung, head and blood. Excretion was studied by whole body counting and measurement of urine and feces. The results showed an initial accumulation in the liver and a later concentration of activity in the lower abdomen, but no marked accumulation of tracer in other organs. In the blood the activity increased steadily for nine hours and then remained constant for 48 hours; formed blood elements contained half as much activity as plasma. Over a period of six days, 50% of the tracer was excreted, 91% of it via urine. The results described agree only in part with those found with LSD in animals by other authors.

Zusammenfassung

Die Verteilung von ^{82}Br-markiertem BOL im menschlichen Organismus wurde durch Messungen der Radioaktivität in Leber, Milz, Intestinal-Trakt, Lunge, Gehirn und Blut untersucht. Die Ausscheidung der injizierten Dosis

wurde in Urin und Faeces und durch «Gesamt-Körper-Messung» verfolgt. Die Untersuchungen ergaben, daß nach einer kurzzeitigen Anhäufung in der Leber und einer späteren Konzentrierung des Tracers im unteren Abdomen keine auffallenden Aktivitätsmengen in anderen Organen auftraten. Im Blut stieg die Radioaktivität über eine Zeitspanne von 9 Stunden ständig an und blieb dann für die nächsten 48 Stunden konstant. In den Blutzellen betrug die Aktivität nur etwa die Hälfte der des Plasmas. Innerhalb von 6 Tagen wurden 50% der injizierten Dosis vom Körper ausgeschieden, davon 91% im Harn. Die beschriebenen und diskutierten Ergebnisse stimmen nur teilweise mit den von anderen Autoren im Tierexperiment gewonnenen Resultaten überein.

Résumé

Dans une étude du ^{82}Br-BOI chez l'homme, la distribution dans le temps a été étudiée dans le foie, la rate, les intestins, les poumons, la tête et le sang. L'excrétion a été étudiée en comptant l'organisme en entier et mesurée dans les urines et les matières fécales. Les résultats démontrent une accumulation initiale dans le foie et une concentration consécutive de l'activité dans la partie basse abdominale, mais aucune autre accumulation n'est présente dans les autres organes. Dans le sang, l'activité augmente constamment pendant 9 heures et reste constante après pendant 48 heures ; les éléments sanguins contiennent la moitié de l'activité du plasma. Pendant 6 jours 50 % du produit a été excrété dont 91 % par voie urinaire. Les résultats décrits confirment seulement en partie ceux que d'autres auteurs ont trouvés chez l'animal avec le LSD.

Acknowledgments

The authors would like to express their gratitude to Dr. Hiroshi Saito, Mr. Helmut Pohlit, Mrs. Elsa Isaac and Mrs. Anne Low-Beer for their invaluable assistance and enthusiastic cooperation. The precursor compound for the synthesis of the ^{82}Br-BOL was kindly supplied by Dr. J. Rutschmann, Sandoz A.G., Basle, Switzerland.

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