of 1 μg. Other estimates of the absorption have been made in man, using the insoluble chromic oxide (Kreula, 1947; Irwin and Crampton, 1951). These investigators stated that it was virtually unabsorbed, but they were given 4–15 g. and hence the absorption of μg. quantities could not be detected.

In four of the five patients presented here the corrected ^{51}Cr concentration in the blood at the end of the investigation was between 1 and 6% lower than the normal ^{51}Cr value. It is reasonably certain that this discrepancy was due to reabsorption of some of the ^{51}Cr by the gut. It would be difficult to correct precisely for this absorption, because the amount absorbed probably varies with the site of bleeding—that is, there would be greater absorption after bleeding into the stomach than after bleeding into the descending colon. It remains possible that the apparent slight diminution of ^{51}Cr survival in the blood was due to a haemolytic process. However, the process would have had to be very mild. Thus, in patient G. B., in whom ^{51}Cr curves showed the greatest departure from normality, the apparent loss of red cells was only 0.25% a day, whereas in haemolytic anaemia of clinical significance the loss is from 3 to 20% a day.

Roche *et al.* (1957) found that the excretion of ^{51}Cr in the stools in 10 subjects without evidence of gastro-intestinal bleeding was equivalent to 1.27 ml. of blood a day. As their subjects had approximately normal haemoglobin concentrations, this amount of blood probably represented about 0.5 ml. of red cells. The quantities found in the two subjects studied here were less than this—namely, 0.1 and 0.2 ml. of cells a day. Roche *et al.* did not wash the red cells after labelling with ^{51}Cr, and hence approximately 10% of the injected ^{51}Cr was present in the plasma. This unattached ^{51}Cr may account for their higher normal values. Owen *et al.* (1954) found a greater amount of ^{51}Cr in the stools after the injection of sodium chromate intravenously than after the injection of ^{51}Cr-labelled red cells. In the present investigation the red cells were washed free from unabsorbed ^{51}Cr before injection.

Owen *et al.* (1954) have also shown that ^{51}Cr is excreted in the stools after the intravenous injection of trivalent ^{51}Cr (red cells are not labelled by trivalent ^{51}Cr); thus the ^{51}Cr found in the stools in normal subjects could be derived either from the ^{51}Cr which elutes out of surviving red cells or from that which is freed from cells destroyed within the body. On the other hand, there may be a small loss of red cells into the gut each day.

Both the ^{51}Cr-labelling and the occult blood tests using benzidine are unsatisfactory when only small amounts of red cells are lost in the stools. The occult blood tests using the peroxidative activity of haemoglobin are limited, because if they are made too sensitive other peroxidases present in faeces will give false-positive results. The ^{51}Cr-labelling method is limited because ^{51}Cr is normally excreted in the stools after the injection of labelled cells. Needham and Simpson (1952) recommended the use of 0.5% benzidine solution for the occult blood tests, and they found that it was necessary to give approximately 2 ml. of cells by mouth before a positive occult blood test could be obtained. The use of ^{51}Cr will enable 2% of this amount of red cells to be detected—that is, 0.04 ml. of cells (see method 2)—but this advantage will be offset by the normal excretion of ^{51}Cr, equivalent to 0.1–0.5 ml. of cells a day. Hence the ^{51}Cr method is useful only when the chromium excreted exceeds the equivalent of 0.5–1 ml. of red cells a day.

Summary

Blood loss in the stools can be estimated quantitatively by labelling a sample of the subject's red cells with radioactive chromium (^{51}Cr), reinjecting the cells into the circulation, and subsequently estimating the ^{51}Cr content of the stools. This method of investigation was used in six patients known to be bleeding from the gastro-intestinal tract. As expected, the rate of loss of ^{51}Cr from the blood stream was increased, and this was almost completely accounted for by the amount of ^{51}Cr lost in the stools. The fact that the amount of ^{51}Cr in the stools did not completely account for the rate of loss of ^{51}Cr from the blood stream indicates that some ^{51}Cr is reabsorbed from the gastro-intestinal tract (but is not reutilized). Thus the ^{51}Cr method slightly underestimates the amount of blood lost in the stools.

In two control subjects who were thought not to be bleeding into the gastro-intestinal tract, the amount of ^{51}Cr excreted in the stools following the intravenous injection of ^{51}Cr-labelled cells was very small (equivalent to 0.1 to 0.2 ml. of red cells a day). Because of this slight excretion of ^{51}Cr in the stools of normal subjects whose cells have been labelled with ^{51}Cr, the daily loss of blood in the stools must exceed 1 ml. a day before it can be detected by this method.

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EFFECT OF RESERPINE IN THERAPEUTIC DOSAGE ON EXCRETION OF 5-HYDROXYINDOLEACETIC ACID

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Recent investigations into the biochemistry of 5-hydroxytryptamine (H.T.) have provided evidence that the endogenous H.T. in the mammalian brain is mobilized and disappears from the organ following the injection of reserpine (Brodie *et al.*, 1955); the hypothesis has been advanced that the latter drug owes its pharmacological properties, hypotensive and ataractic, to its ability to set free H.T. or, as an alternative, to interfere with the H.T.-binding sites, the immediate liberation being incidental.

Reserpine will also set H.T. free from blood platelets *in vitro* (Shore *et al.*, 1956) and *in vivo* in animals and man (Haverback *et al.*, 1956a), but the stores of this compound in the intestinal cells are not so readily liberated, although this has been reported following high dosage in the rabbit (Pletscher *et al.*, 1955). A resultant increase in the excretion product, 5-hydroxyindoleacetic acid (H.I.A.A.), has been demonstrated in the dog following massive doses of reserpine; the dose used, 5 mg./

kg., was 50–100 times the therapeutic dose (for ataraxis) in man (Shore *et al.*, 1955).

In view of the importance of reserpine in psychiatry, it appeared desirable to investigate whether these changes could be observed in man following treatment with therapeutic dosages of the drug. Failure to demonstrate such changes could be regarded as reducing the probability of the hypothesis mentioned above.

Of the possible alternatives, the excretion of H.I.A.A. was chosen on technical grounds as the first subject for study.

Haverback *et al.* (1956b) have reported no significant increase in H.I.A.A. excretion by six individuals receiving reserpine, but the conditions differed from those used in the present study. More recently there have been preliminary reports of an increase in H.I.A.A. excretion following reserpine (Fraser *et al.*, 1957 ; Valcourt, 1957).

Methods

The procedures and techniques used have been modified in the course of the investigation. Those described here were employed throughout the final series of tests, which are the only ones reported in detail.

Plan of Test.—All patients were males from an admission ward, the staff of which had previously collaborated in the preliminary studies. It was planned that cases should consist, so far as possible, of two equal groups of (*a*) schizophrenics and (*b*) neurotics and psychopaths ; the diagnosis was unknown to the laboratory until the completion of the investigation. Each patient was kept in bed for the duration of the test ; the bladder was emptied at 10 p.m. on the evening before the injection was to be given, and all urine was then collected separately over the following periods: 10 p.m.–7 a.m. ; 7–11 a.m. ; 11 a.m.–3 p.m. ; 3–7 p.m. ; 7–10 p.m. Reserpine (" serpasil "), 5 mg., was injected intramuscularly immediately following the completion of the 7–11 a.m. collection. Specimens were collected in brown glass bottles containing 1–2 ml. of concentrated hydrochloric acid (Scandrett, 1956). They were received in the laboratory within five hours of the end of the collection, brought to *p*H 3, and stored overnight in a refrigerator for analysis next morning.

Estimation of 5-Hydroxyindoleacetic Acid

After the brief initial report that H.I.A.A. could be estimated by its reaction with 1-nitroso-2-naphthol (Udenfriend *et al.*, 1955a), the possibilities of this reaction were studied ; a second compound producing a colour with the reagent was detected chromatographically in some urines. Separation of this chromogen and examination of the absorption spectrum of the coloured reaction product suggested that it would not interfere if the optical density of the colour from H.I.A.A. were read at 640 mμ instead of 540 mμ.

The method of Udenfriend *et al.* (1955b) was later adopted in essence, but the modification of reading at 640 mμ was retained, the chloroform extraction being omitted. In addition, since the psychiatric cases under investigation did not include any with gross disorders of carbohydrate metabolism, the treatment with acid 2,4-dinitrophenylhydrazine solution was also omitted. This permitted the doubling of the ratio of urine to ether in the extraction which compensated for the 50% loss of sensitivity incurred by reading at 640 mμ.

However, following the observation (see below) that in concentrated urines the whole of the H.I.A.A. was not estimated, the procedure was adopted of diluting the urines more or less in proportion to their concentration by taking as aliquots of the specimens a fixed fraction (one-tenth, equal to a six-minute volume) of the hourly output. Thus, for a four-hour specimen of 600-ml. volume, 15 ml. would be taken, while for an overnight (nine-hour) specimen of 270-ml. volume 3 ml. would be taken. Before extraction these were diluted to a final volume of 20 ml. with distilled water acidified to *p*H 3.

A preliminary estimation was carried out on the overnight specimen, which was both the first available and one of the more concentrated ones ; 3-, 6-, and 9-ml. portions of urine were diluted and the H.I.A.A. was estimated. In 7 out of the 10 cases the estimate based on the 6-ml. specimen was 90% or more of that based on the 3-ml. specimen (mean 95%) ; the actual volume subsequently taken on the six-minute aliquot basis averaged 3.9 ml.

Estimations were carried out in duplicate ; where the difference between duplicates exceeded 10% the estimation was repeated.

Estimation of Creatinine

Creatinine was estimated by the method of Bonsnes and Taussky (1945) as described by Varley (1954), except that the volume of urine taken was not fixed but was related to the volume of the specimen. Where duplicates estimated at different times differed by more than 4% a repeat estimation was carried out.

Experimental Results of Preliminary Investigations

The first series of tests consisted in measuring the 24-hour excretion of H.I.A.A. by patients due to start a course of reserpine therapy. This was continued from three days before the start to about a week afterwards and at intervals

TABLE I.—*Individual Excretion of H.I.A.A. and Creatinine Before and After Reserpine (5 mg. of Reserpine Injected Intramuscularly at 11 a.m. in Every Case)*

Case No.	Period	Urine Volume (ml./hr.)	Creatinine (g./hr.)	H.I.A.A. (mg./hr.)	H.I.A.A./Creatinine Ratio (mg./g.)	% Change from Pre-Reserpine Mean	Mean % Change after Reserpine*
1	10 p.m.–7 a.m.	29	0·0625	0·182	2·92	+ 3	± 0
	7–11 a.m.	46	0·0748	0·205	2·74	− 3	
	11 a.m.–3 p.m.	92	0·0536	0·163	3·04	+ 8	
	3–7 p.m.	280	0·0716	0·213	2·97	+ 5	
	7–10 p.m.	69	0·1089	0·254	2·33	−18	
2	As above	34	0·0546	0·134	·2·46	+ 1	+25
		50	0·0643	0·155	2·41	− 1	
		37	0·0609	0·236	3·88	+60	
		(10	0·0273	0·072	2·64	+ 8)	
		39	0·0721	0·179	2·48	+ 2	
3	,, ,,	29	0·0611	0·189	3·10	+ 5	+53
		189	0·0685	0·194	2·83	− 5	
		284	0·0707	0·362	5·12	+73	
		113	0·0687	0·322	4·69	+59	
		207	0·0797	0·276	3·46	+17	
4	,, ,,	66	0·0758	0·287	3·79	− 2	+ 1
		74	0·0809	0·316	3·91	+ 2	
		94	0·0610	0·298	4·89	+27	
		66	0·0845	0·295	3·49	− 9	
		43	0·0501	0·156	3·11	−19	
5	,, ,,	21	0·0481	0·138	2·87	+ 3	+46
		35	0·0421	0·114	2·71	− 3	
		109	0·0578	0·275	4·77	+71	
		16	0·0582	0·239	4·11	+48	
		25	0·0693	0·214	3·09	+11	
6	,, ,,	77	0·0603	0·246	4·08	+ 3	+ 6
		198	0·0628	0·241	3·83	− 3	
		256	0·0734	0·416	5·67	+44	
		61	0·0827	0·271	3·28	−17	
		123	0·0804	0·278	3·45	−13	
7	,, ,,	58	0·0580	0·208	3·59	+ 1	+51
		118	0·0796	0·281	3·53	− 1	
		149	0·0633	0·388	6·13	+72	
		38	0·0699	0·407	5·82	+64	
		59	0·0922	0·352	3·82	+ 7	
8	,, ,,	150	0·0848	0·126	1·49	−24	+ 7
		63	0·0545	0·133	2·44	+24	
		183	0·0548	0·121	2·21	+12	
		77	0·1149	0·261	2·27	+16	
		59	0·0802	0·142	1·77	−10	
9	,, ,,	29	0·0831	0·481	5·79	+21	+39
		30	0·0491	0·186	3·79	−21	
		61	0·0541	0·384	7·09	+48	
		2	0·0043	0·009	6·43	+34	
		27	0·0946	0·626			
10	,, ,,	55	0·0666	0·194	2·92	+ 4	+ 6
		174	0·0610	0·163	2·67	− 4	
		146	0·0735	0·184	2·50	−11	
		80	0·0548	0·182	3·32	+19	
		37	0·0748	0·232	3·10	+11	

* Weighted to allow for difference in lengths of periods.

subsequently. The patients were not subject to any special degree of control, and losses of urine were suspected, since the volumes collected varied considerably from day to day.

The average excretion of H.I.A.A. in the group appeared to be slightly above the average on the first day of the investigation and on the first day after reserpine; this indicated that the reliability of the collection declined after the first 24 hours, but also suggested that there might be a slight increase in H.I.A.A. excretion following the first dose of reserpine.

In an attempt to obtain more positive results, tests were carried out on four normal subjects, each given a single dose of 5 mg. of reserpine. The plan of the collection differed only in detail from the standardized test subsequently adopted. In the last of these the estimation of creatinine as well as H.I.A.A. was introduced, following the principle of Snow *et al.* (1955). An increase in H.I.A.A. excretion was observed in three out of the four cases; the relating of the H.I.A.A. to creatinine gave the response a more regular form (see below). This series was then completed by tests on six patients.

A review of the results indicated that 6 out of these 10 subjects had shown increased excretion of H.I.A.A. following reserpine, particularly during the first four hours. These had also exhibited a marked diuresis, while others had not. Tests were therefore carried out to determine whether the latter could by itself account for the rise in H.I.A.A. They indicated that it could not, but at the same time demonstrated that the method of estimation was not recovering 100% of the H.I.A.A. from concentrated urines. This point was investigated in some detail, and fully confirmed, without any specific cause being found; however, adequate dilution appeared to provide a practical solution to the problem and the method described in the previous section of taking a fixed fraction of the urine specimen was finally adopted.

Results in Final Series

The results of the tests on the final series of 10 selected patients are given in Table I. The hourly rate of urine excretion varied from 2 to 284 ml. in the group; even neglecting the smallest specimen, there were two rates of under 20 and four of over 200 ml./hr., and the individual ranges in rate exceeded fourfold in the majority of cases, being nearly tenfold in two.

It had previously been noticed that reserpine appeared to cause a diuresis which was followed by a reduction of the fluid output to an inconveniently low level in the later stages of the test; it was therefore arranged that patients should be given ample fluid during the afternoon. However, the weather during the investigation included a number of unusually hot days which caused an abnormally high loss of water by perspiration in some cases (in particular in the period for which the 2 ml./hr. rate was observed). Accidental loss (about 10 ml.) was reported on one occasion.

The means and ranges of the volumes excreted in the several periods of the test are given in Table II.

TABLE II.—*Rates of Urine Excretion*

Period	Mean Rate of Excretion (ml./hr.)	Range
10 p.m.–7 a.m.	54·8	21–150
7–11 a.m.	97·7	30–198
11 a.m.–3 p.m.	141·1	37–284
3–7 p.m.	74·3 (91·4)*	2–280 (16–280)*
7–10 p.m.	68·8	25–207

* Omitting two lowest figures (see below).

Diuresis, therefore, was again observed in the period following the injection of reserpine. There was a tendency for the rate of urine excretion to be somewhat reduced in the second and third periods despite the measures taken to maintain it.

Low rates of excretion could reduce the total amount of solids excreted during a period, including the amounts of creatinine and H.I.A.A. Since creatinine excretion is regarded as being fairly steady under normal conditions, and since, as is shown above, the variations during the 24 hours were not great, the creatinine excreted in small volumes has been compared with that excreted in the other periods by the same individual; in the 2 and 10 ml./hr. specimens the amounts were abnormally low, but in the next smallest specimens, 16 and 21 ml./hr., the amounts excreted were within the range for the individual.

The creatinine excretion had been measured in the first instance as a check on the reliability of the urine collection and subsequently in order to be able to determine the H.I.A.A./creatinine ratio, which appeared to improve the regularity of the results. Any change in this ratio could be due to a change in either the H.I.A.A. or the creatinine excreted, or in both; the figures for creatinine have therefore been analysed to determine to what extent their variation could account for any alteration of the ratio. The mean creatinine levels in each period are shown in Table III. The figures show that there is a negligible fall in the mean creatinine level in the first period after reserpine, by comparison with the observed rises in the H.I.A.A./creatinine ratio, and that during the second and third periods there is an actual rise in the creatinine level which would, in fact, tend to reduce the ratio.

TABLE III.—*Mean Creatinine Excretion*

Period:	10 p.m.–7 a.m.	7–11 a.m.	11 a.m.–3 p.m.	3–7 p.m.	7–10 p.m.
Creatinine excretion (mg./hr.)	65·5	63·9	62·4	75·9	80·2
,, ,, —percentage change	—	—	−4	+17	+24

There remains, however, the possibility that the patients showing an increase in H.I.A.A./creatinine ratio in response to reserpine do so by reason of their exhibiting an equal and opposite change in creatinine excretion from the unresponsive patients. An analysis of this possibility is given in Table IV.

TABLE IV.—*Creatinine Excretion According to Response to Reserpine*

Period:	10 p.m.–7 a.m.	7–11 a.m.	11 a.m.–3 p.m.	3–7 p.m.	7–10 p.m.
Positively responding Group (4):					
Creatinine (mg./hr.) ..	62·5	60·0	61·5	65·7	84·0
,, —percentage change	—	—	±0	+7	+37
Unresponsive Group (5):					
Creatinine (mg./hr.) ..	70·2	67·0	63·4	82·0	78·8
,, —percentage change	—	—	−8	+19	+15

While the numbers in each group are small, it seems that, owing to changes in creatinine output, the ratio for the unresponsive group would increase more than that for the positively responding group during the first four hours after reserpine; this would be counterbalanced by a smaller decrease in the ratio for the positively responding group during the second four hours; the overall effect favours a relative rise in the ratio of the unresponsive group. The observed trends in H.I.A.A./creatinine ratio are therefore quite independent of changes in creatinine excretion following reserpine.

It has previously been suggested that the analysis of the results in terms of the H.I.A.A./creatinine ratio rather than in terms of H.I.A.A. excreted per hour leads to a greater regularity in the results. *A priori*, the actual hourly excretion might be preferred on the grounds that it is a direct estimate, but the ratio does make allowance for one possible and not easily recognizable cause of error—namely, loss or retention of urine.

One test of the alternative methods of handling the data has been to compare the estimates obtained from the two pre-reserpine periods. Four times there were considerable

differences between these estimates, twice with each method; these involved three cases. In the remaining seven cases the differences were relatively small, but the mean percentage difference in H.I.A.A. per hour was 10.9, whereas the mean percentage difference in the ratio was less than two-thirds of this figure (6.0).

The maximum increase in H.I.A.A per hour after reserpine occurs five times in the first period, twice in the second, and thrice in the third, whereas the maximum increase in the ratio occurs eight times in the first and twice in the second period.

The mean percentage increase in H.I.A.A. excretion for the whole 11 hours following reserpine has been calculated by both methods, and is given in Table V.

TABLE V.—*Mean Percentage Increase in H.I.A.A. Excretion Following Reserpine*

Case No.:	1	4	10	6	8	2	9	7	3	5
Mean percentage increase:*										
5-H.I.A.A.(mg./hr.) ..	−17	+9	+13	+32	+35	+46	+52	+57	+68	+94
,, (mg./g. creatinine)	±0	+1	+6	+6	+7	+25	+39	+51	+53	+46
Classification of response	−	−	−	−	−	±	+	+	+	+

* For three periods following reserpine injection.

The mean percentage change in H.I.A.A. excretion per hour forms a broad spectrum of values, while the H.I.A.A./creatinine ratio figures fall more clearly into two groups, the absolute values of which, 0 to +7 and +39 to +53, can justifiably be equated to negative and positive responses. Division of the H.I.A.A./hr. figure into two types of response would be possible, but more than one arbitrary division could be made.

The H.I.A.A./creatinine ratio having been adopted, the results of the tests on the response of humans to reserpine can be summarized as follows. There have been no examples of significant depression of H.I.A.A. excretion following reserpine, but about half the cases have responded to 5 mg. intramuscularly by a clear-cut increase in H.I.A.A. excretion.

The fact that the response is not always present is evidence against any suggestion that H.I.A.A. excretion normally rises after midday; there is also direct evidence to indicate that it varies little throughout the 24 hours (Table VI).

TABLE VI.—*Diurnal Variation in H.I.A.A. Excretion (mg./hr.)*

Subject	7–11 a.m.	11 a.m.–3 p.m.	3–7 p.m.	7–11 p.m.	11 p.m.–7 a.m.
A* ..	0·29	0·25	0·25	0·22	0·21
B ..	0·22	0·21	0·24	0·25	0·24
C ..	0·26	0·26	0·28	0·29	0·25

* Collection in this case was 8.30–8.30 a.m.

This investigation was concluded by comparing the response of the patient to reserpine with the psychiatric diagnosis and with certain other psychiatric and clinical parameters. It has been considered justifiable to include the previous series of cases in this comparison, since the experimental data are regarded as probably adequate in most instances for the assessment of positive or negative responses.

TABLE VII.—*Comparison of Response to Reserpine with Diagnosis*

	Response	
	Negative	Positive (and Doubtful)
1st series	Normal	Normal
	Schizophrenic	,,
	,,	,,
	Depressive	Schizophrenic
		Chronic anxiety state
		Inadequate psychopath (? M.D.)
2nd series	Schizophrenic	Schizophrenic
	,,	,,
	Aggressive psychopath	,,
	Alcoholic	Aggressive psychopath
	,,	Anxiety state

From Table VII it can be seen that these responses did not differentiate between schizophrenic and non-schizophrenic diagnoses. Similarly, the response was not related to subtype of schizophrenia, to primary thought disorder, or to gross secondary symptoms in schizophrenia, nor, in the group as a clinical whole, to age, height, weight, height/weight ratio, blood pressure, duration of psychiatric process, or the presence of symptoms of tension.

Discussion

Our preliminary observations broadly confirmed those of Haverback *et al.* (1956b) that there was no significant rise in the 24-hour urinary H.I.A.A. excretion following reserpine. Subsequent tests, however, indicated that there was a rise in H.I.A.A. excretion in about half the cases which was maximal in the first four hours after the injection and had fallen to about one-sixth of the maximum between the eighth and eleventh hours. It is not possible to say whether the excretion would later have fallen below normal, though it is perhaps worth noting that two of the five cases showing negative responses did so as the result of a transient rise being counterbalanced by lower than normal excretions during the second and third periods.

In the animal experiments the 400% increase observed was regarded as coming largely from the intestinal stores of H.T. (Shore *et al.*, 1955); since these are thought to be more resistant to mobilization by reserpine than other sources (platelets, brain, spleen) it is of interest to calculate whether the platelet H.T. could adequately account for our observed increase in H.I.A.A.

In the group showing a positive response, the mean increase in H.I.A.A. excretion over the control level totalled 1.2 mg. for the 11-hour period. There was a slight increase in the unresponsive group, 0.2 mg., which could be deducted, leaving 1 mg. to be accounted for.

A recent figure for the H.T. content of whole blood is 0.16 ± 0.06 μg./ml. (Hardisty and Stacey, 1955); on the assumption that the blood volume is 8% of the body weight, this gives the normal range for the total blood H.T. as 0.8 ± 0.3 mg.

Summary

The H.I.A.A. excretion of four normal subjects and 16 mental patients has been studied before and after an intramuscular injection of 5 mg. of reserpine.

An increase was observed in half the subjects; this was maximal and of the order of 60% of the control level over the first four hours; it remained high during the second four hours; but thereafter was hardly significant.

The responsiveness or otherwise of the patients to the drug does not correlate with the psychiatric diagnosis.

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