important factor in the control of tuberculosis. Early diagnosis of the disease is of the utmost value. Mass radiography, substantially on a voluntary basis, is the medium in human medicine. Tuberculin-testing is the counterpart in the veterinary field, and voluntary measures are rapidly giving way to compulsion in relation to cattle. I think it is safe to say that the cattle of Britain will be free from this scourge before the people, but there will remain other animals to keep the disease alive in its various forms.

Summary

The proper study of tuberculosis among domestic animals started during the period of the industrial revolution, which played a great part in the increase of tuberculosis in man and animals, particularly in overcrowded towns. Although infection in both man and animals is via the respiratory tract and the alimentary canal, the lung and regional lymph-node lesions are not convincing evidence of aerogenous infection, as the tubercle bacillus can pass through the intestinal wall and localize in the pulmonary tissues without leaving any trace of its path.

Tuberculosis of cattle is nearly always caused by tubercle bacilli of bovine type; human and avian tubercle bacilli have rarely been found in cattle. At present, of the total of over ten million cattle in Great Britain, 73% are free of tuberculosis, and it is hoped that in the very near future tuberculosis will be completely eradicated from them. The part played by ruminants other than cattle and horses in spreading infection is slight, as they are rarely infected; but so long as only a few infected animals exist there will always be a hazard of infection.

Pigs are susceptible to human, bovine and avian types of tubercle bacilli, but the bovine type is most common. The incidence of tuberculosis in pigs varies from 10 to 15%. The poor sanitary standard of slaughterhouses and the handling of meat and organs of tuberculous animals present a considerable risk of infection for meat inspectors and slaughterhouse employees.

Dogs and cats are a great risk in the dissemination of tuberculosis. At present there is no legislation by which a tuberculous dog or cat can be removed from the family. Therefore it is suggested that a veterinarian should be attached to the Ministry of Health in order to advise on the diseases transmitted from animals to man.

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INDOLIC COMPOUNDS IN THE URINE OF SCHIZOPHRENICS

BY

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It has been known for many years that certain drugs, such as harmine, ibogaine, hashish, and mescaline, were capable of giving rise to hallucinations in doses which were not dangerous to life and produced only minor physical effects. More recently the range of hallucinants has been increased by the synthesis of lysergic acid diethylamide, medmain, adrenochrome, etc. These new drugs are much more active, and will give rise to a well-developed drug psychosis in doses not producing more than a very transient vasomotor or peripheral neurological discomfort. Osmond and Smythies (1952) and Hoffer *et al.* (1954) pointed out that all these drugs contained or could probably be easily converted into a chemical containing an indole nucleus. Woolley and Shaw (1954) also commented on the similarity of many of these substances to serotonin and on their ability to antagonize the action of serotonin on the arterial wall. On the other hand, most of these chemicals have another common factor—namely, an ethylamino or diethylamino side-chain directly attached to the benzene or pyrrole ring—so that the pharmacological evidence cannot unequivocally incriminate disordered indole metabolism as a cause of the hallucinations.

Turning to clinical evidence, Riegelhaupt (1956) found that there was an excess of a substance giving the glyoxylic reaction (Hopkins and Cole, 1901) in the urine of many more schizophrenics than in those patients suffering from other forms of mental disease. The glyoxylic reaction was at one time thought to be specific for tryptophan, but it is now known to be given by quite a number of chemicals containing an indole nucleus. For these reasons it seemed worth while to make a detailed study of the indolic compounds found in urines of schizophrenics and to compare them with those found in normal controls. This was made a practical possibility by the work of Dalgliesh (1955) and Jepson (1955). Their methods were combined and adapted in the following manner for the present investigation.

Experimental

From the first specimen of urine passed in the morning 200 ml. was collected. The creatinine was estimated by the method of King and Wootton (1956) and a concentrate made by the technique described by Dalgliesh (1955), except that the carbon was blocked with 8% *n*-octadecylamine. During the steam distillation under reduced pressure the temperature was not allowed to rise above 65° C., since higher temperatures made the semi-quantitative results less repeatable. This was particularly noticeable with indole compounds substituted in the benzene ring. The final concentrate was dissolved in 50% v/v mixture of isopropyl alcohol and water. The volume of this solvent was so arranged that the aromatic substances recovered from a volume of urine containing 100 mg. of creatinine were present in 1 ml. of the final concentrate. This concentrate does not keep for long without decomposition, even at -15° C., and so it was applied as soon as possible to sheets of Whatman's 3 MM filter paper 34 by 32 cm. The volume

used was such that the equivalent of 2 mg. of creatinine was represented in the "spot," which was kept less than 0.7 cm. in diameter.

The "spotting" was carried out at room temperature without air blast or heat, both of which procedures were found to detract from the accuracy of the results. The papers were transferred to all-glass chromatograph tanks. The first dimension was run in a mixture of isopropyl alcohol 100 parts, water 10 parts, and ammonia (sp. gr. 880) 10 parts at room temperature for 18 hours. The papers were dried in an oven maintained at 40° C. and, as soon as they were dry, transferred to a refrigerator, where they were kept until the evening, when they were run in the second dimension in *n*-butyl alcohol 600 parts, acetic acid 200 parts, and water 250 parts. Again the run was overnight (usually 16 hours) in a refrigerator which maintained a temperature between 0 and 4° C., the runs in each case being in the ascending direction without equilibration. The papers were dried as before and immediately sprayed with Ehrlich's reagent (1% solution of *p*-dimethyl-aminobenzaldehyde) in 2N HCl. The papers were hung at room temperature for exactly three hours and the maximum density of the "spots" was read without the use of a colour filter on a photo-electric densitometer, the galvanometer of which was provided with a logarithmic scale.

Although this technique cannot be used at all easily to measure the actual quantity of the indoles present, it is a very satisfactory method of comparing the amounts of indolic substance present in a number of specimens, since it gives both consistent and reproducible results. In artificial mixtures of indolic compounds, in normal urine, and in urines to which indolic compounds have been added, the density of the "spot" bears a fixed non-linear relation to the amount of the substance present in the original specimen.

After much preliminary work it was decided to investigate in detail seven well-defined spots which occurred fairly consistently on the chromatograms made from the urinary concentrates of schizophrenics. The chemicals causing five of these spots are known beyond reasonable doubt; they are tryptophan, indolyl acetic acid, 5-hydroxy-indolyl acetic acid, indolyl acetyl glutamine, and potassium indoxyl sulphate. One of the other two has been provisionally identified as potassium skatoxyl sulphate, since a substance with exactly similar chromatographic properties can be obtained from an alkaline persulphate oxidation of skatol; nevertheless this will be referred to as spot "Q." It has an R_f of 0.79 in the isopropyl alcohol solvent and of 0.43 in the *n*-butyl alcohol solvent. The other substance will be referred to as spot "S." It has R_f values of 0.31 and 0.44 respectively in the two solvents. With Ehrlich's reagent it gives a blue-violet colour, which appears rapidly and fades only slowly. Hydrolysis of the urine has no effect on the depth of the spot. It does not stain with diazotized I.C.I. 5901 ("Boscott's reagent," Boscott and Cooke, 1954) nor with 1-nitroso-2-naphthol. These reactions suggest that, while the substance causing this spot almost certainly contains an indole nucleus, it is not a 5-hydroxy derivative of tryptophan. These seven spots were by no means all those on the chromatograms of the urine concentrates that were observed to stain with Ehrlich's reagent in a manner suggestive of an indole compound, but they were the most constant and discrete observed.

Selection of Patients and Controls

Fifty "confirmed" schizophrenic patients between the ages of 19 and 40 were selected from a much larger number of possible examples in the wards of Hollymoor and Rubery Hill Hospitals. All conformed to the following criteria: (1) They had been in hospital long enough for their notes to show that they suffered from at least four of the following symptoms, considered by many psychiatrists to be typical of schizophrenia: "thought disorder, withdrawal of interest from surroundings, hallucinations, retardation, incongruity of affect, attitude of suspicion, delusions, disorientation,

morbid introspection." (2) They had all been diagnosed as schizophrenics by the consultant psychiatrist in charge of the ward in which they were during the period of this investigation. (3) They were either not receiving active treatment or could be taken off treatment for the period of the investigation.

There were 28 males and 22 females, and their average age was 27 years. Fig. 1 shows the length of time symptoms had been present and the number of patients in each group.

The controls consisted of members of the nursing staff of the two hospitals. Their average age was 31 years, and there were 23 males and 27 females. The male-female ratio in the controls is slightly lower than in the group of patients, but this is not regarded as important, since no difference between the results for the two sexes could be detected in either group. Although it is not considered likely that small variation in diet affects appreciably the substances being investigated, an effort to eliminate this possible source of error was made by collecting the urine of the controls and patients after they had received similar diets. It was not found possible to prevent the patients having any drugs at all, since barbiturates and other hypnotics are often essential. No patient who had received treatment recently with any of the tranquillizers was included in the investigation. The concentrates from the patients and controls were made and the chromatograms run alternately whenever possible in order to minimize the effect of day-to-day variations in conditions and technique.

FIG. 1.—Duration of illness.

Results

Figs. 2 to 8 show the results in diagrammatic form. The densitometer readings for this purpose are shown in 10-unit grouping.

From Fig. 2, which portrays the excretion of 5-hydroxy-indolyl acetic acid, it is obvious that there is a considerable difference in the distributions as between the control group and the schizophrenics. None of the control group shows a densitometer reading below 10, while 11 out of 50 of the schizophrenics gave this result. There is also more scatter for the readings of the schizophrenics, there being about an equal number of cases in each group from 0 to 40, while more than half the normal controls fall between 20 and 30. Fig. 3 shows the excretion of the substance designated as spot S. The diagram demonstrates clearly that the schizophrenics excrete much more of this substance than do the controls. Almost three-fifths of the controls have a reading of less than 10, while four-fifths of the schizophrenics have a reading of 10 or more. Fig. 4 shows the results for spot Q (which may well be potassium skatoxyl sulphate). Here again the schizophrenic group excrete more than the control group, but the difference is somewhat less striking.

Figs. 5, 6, 7, and 8 show the excretion of tryptophan, indolyl acetic acid, potassium indoxyl sulphate, and indolyl acetyl glutamine respectively. For these substances not only is there no outstanding difference between the two groups but the pattern of excretion is moderately similar. It is noteworthy that the results for potassium indoxyl sulphate are not higher for the schizophrenics than for the normal controls.

Table I shows these results analysed both by the standard error of the differences between the means and by the χ^2 test (using a 2 by 3 or 2 by 4 table after grouping, so that the expected result for any cell is not less than 5) on the overall distribution of results set out in Figs. 2 to 8.

An examination of Table I shows that the numerical results confirm the impression gained from the diagrams. For spot S the difference between the normals and schizophrenics is highly significant. For 5-hydroxy-indolyl acetic acid and for spot Q the differences exceed the accepted

levels of "significance" ($P < 0.05$) as judged by one or other of the tests. The similarity of the control group and the schizophrenics for the other four substances already demonstrated in the diagrams is supported by the analysis in Table I.

TABLE I

Substance	Control Mean	Schizophrenic Mean	S.E. of Difference	P	χ^2	P
5-Hydroxy-indolyl acetic acid ..	26.7	22.9	2.15	0.1	7.9	0.05
Spot S ..	9.5	22.0	2.3	0.001	12.6	0.01
" Q ..	19.1	29.0	3.37	0.01	6.6	0.1
Tryptophan ..	30.4	28.9	3.19	0.6	2.25	0.5
Indolyl acetic acid ..	24.3	25.7	2.64	0.6	1.67	0.3
Potassium indoxyl sulphate ..	29.6	27.6	2.23	0.3	0.59	0.8
Indolyl acetyl glutamine ..	30.0	28.1	2.85	0.4	1.22	0.7

FIG. 2.—5-hydroxy-indolyl acetic acid.

FIG. 3.—Spot S.

FIG. 4.—Spot Q.

FIG. 5.—Tryptophan.

TABLE II.—Densitometer Readings

Patient	Specimen					Mean	S.D.
	1st	2nd	3rd	4th	5th		
<i>5-Hydroxy-indolyl-3-acetic acid. Normal mean, 26.7.</i>							
<i>Standard deviation, 9.2</i>							
TU	11	7	6	11	10	9.0	2.1
JA	9	12	8	6	10	9.0	2.1
DI	6	8	7	9	8	7.6	1.0
MA	8	9	6	10	7	8.0	1.1
JM	8	11	6	12	10	9.4	2.1
<i>Spot S. Normal mean, 9.5. Standard deviation, 10.6</i>							
JA	34	33	40	46	37	38.0	4.6
DI	16	23	29	30	22	24.0	5.1
MA	34	33	40	40	47	38.8	4.7
LO	32	36	42	34	30	33.6	4.4
SI	47	37	43	30	41	39.6	5.8
<i>Spot Q. Normal mean, 19.06. Standard deviation, 17.6</i>							
JA	9	10	15	12	16	12.2	2.7
DI	33	40	31	42	33	35.8	4.3
MA	31	42	30	46	37	37.2	6.2
LO	34	37	30	43	33	35.2	4.5
SI	57	46	37	43	32	43.0	8.5

FIG. 6.—Indolyl acetic acid.

FIG. 7.—Potassium indoxyl sulphate.

FIG. 8.—Indolyl acetyl glutamine.

over a considerable period of time. To test this the urine of seven patients was examined on five occasions at approximately fortnightly intervals. Table II shows the results.

These patients were selected because they showed at least two of the three major abnormalities described. Table II shows clearly that during the two and a half months during which specimens were collected there was little change in the level of excretion of the three indolic substances examined. When they were abnormal to begin with they remained outside the normal range and the portion that was normal remained within the normal range. It is quite possible that part of the variation shown in Table II is due to the inherent error in the technique of the estimations, and if this be the case it is probable that the abnormal pattern of excretion of these indole compounds remains even more constant.

Discussion

In attempting to assess the results described it is important to remember two facts. In recent years there has been a growing tendency for schizophrenia to be regarded as a syndrome rather than a single disease. This has made it much more difficult for the investigator interested in any biochemical changes which might be present in schizophrenics, since even if the patients are selected by a group of psychiatrists it is unlikely that they will all be suffering from exactly the same disease, although they may present many of the same symptoms. This difficulty in the choice of patients makes it unlikely that every member of a schizophrenic group would show the same metabolic abnormality.

Also, the chemical technique employed, while capable of demonstrating minute quantities of indole compounds, is only semiquantitative. It follows that small real differences between individual schizophrenic patients and normals could well have been overlooked in the present investigation, although it is realized that they might be important; but in the case of three of the compounds estimated there are major differences to be considered. For the spots S and Q it appears that the majority of schizophrenics excrete more of these substances than the normal. For 5-hydroxy-indolyl acetic acid a proportion in the order of 20% excrete much less than normal. It is realized that the controls in the present series are such as only to show a difference between normals and schizophrenics. It is possible that the differences demonstrated might merely be an association with living in a mental hospital, or might occur equally often in other types of mental diseases, although the work of Rieghelhaupt (1956), who used mentally deranged patients who were not schizophrenics for his controls, makes this unlikely. So far it has not been possible to show any correlation between the abnormalities described, so they must be considered separately.

The group of schizophrenics which has a decreased output of 5-hydroxy-indolyl acetic acid is of special interest because a considerable amount is known about how this final metabolite is produced. It is almost certain that tryptophan is hydroxylated to 5-hydroxy-tryptophan in the argentophil cells of the intestine and probably released into the blood stream (Dalglish and Dutton, 1957; Smith *et al.*, 1957). Where it is decarboxylated into 5-hydroxy-tryptamine (serotonin) is not certain, since 5-hydroxy-tryptophan decarboxylase is widely distributed throughout the body (Gaddum and Giarman, 1956). It is certain that the platelets and certain portions of the brain contain much more 5-hydroxy-tryptamine than most other body tissues (Udenfriend *et al.*, 1955, 1956). Although there is little proof, it is a reasonable assumption that the 5-hydroxy-indolyl acetic acid in the urine is the final oxidation product of 5-hydroxy-tryptamine. It is known that 5-hydroxy-tryptamine is physiologically an extremely active substance; some of its antagonists if given to man give rise to mental aberrations (Woolley and Shaw, 1954), while 5-hydroxy-tryptamine itself if given into the ventricles of the brain gives temporary improvement in cases of schizophrenia (Sherwood, 1955). Unless there is an abnormal deviation from the usual metabolic pathway the

deficiency in the excretion of 5-hydroxy-indolyl acetic acid must be attributed to a lack of the ability to synthesize enough 5-hydroxy-tryptophan. This hypothesis is supported by the fact that by giving 5-hydroxy-DL-tryptophan by mouth it has been found possible, in trials to be described separately, to raise the excretion of 5-hydroxy-indolyl acetic acid of some patients whose urinary excretion of this substance was below normal. Whether this procedure, by which the biochemical abnormality present can be corrected, will be of any value in treatment is still under investigation.

Little can be said about the increase in spots S and Q, as their chemical constitution is not known for certain. Since the amount of potassium indoxyl sulphate excreted is not increased in the schizophrenic group it is very unlikely that bacterial degradation of tryptophan in the intestine plays any part in the finding of excessive quantities of substances S and Q in the urine. Their presence strengthens the case for further investigation into the tryptophan metabolism in schizophrenics.

For the present the difference in depths of spots S and Q between schizophrenics and controls may be of some assistance in the diagnosis of early or obscure cases of schizophrenia. The low level of excretion of 5-hydroxy-indolyl acetic acid would not appear to be suitable for development as a specific test for schizophrenia, since similar findings have been reported in other mental diseases—for example, untreated phenylketonuria (Pare *et al.*, 1957), but it might turn out to be of use to segregate on biochemical lines about 20% of schizophrenics from the remaining 80%. This would be particularly valuable if it were found possible to correlate this biochemical abnormality with other differences in symptoms and prognosis.

Summary

The level of excretion of seven indolic substances found in the urine of schizophrenics is compared with that found in a similar number of normal people. It is found that about 20% of schizophrenics excrete less 5-hydroxy-indolyl acetic acid than any among the control group. Two other substances which probably contain the indole nucleus are excreted in significantly larger quantities by the schizophrenics than by the group of normals.

The significance of the low excretion of 5-hydroxy-indolyl acetic acid is discussed, its possible value as a beginning of a biochemical classification of schizophrenia suggested, and the treatment of certain cases with 5-hydroxy-tryptophan considered.

The possibility of using the excess excretion of the other two indolic substances as an aid to the diagnosis of schizophrenia is mooted.

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