PURINES IN THE URINE OF NORMAL AND SCHIZOPHRENIC SUBJECTS

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ALTHOUGH there have been many attempts to discover in the body fluids, chemical peculiarities characteristic of schizophrenic illness, the outcome of most of these investigations has been inconclusive (see for example Altschule, 1953; Kety, 1959). Little attention has been paid, however, to purine metabolism until the recent studies of Kishimoto (1958) who reported peculiarities in the absorption spectra of body fluids in schizophrenia which he attributed to abnormalities in the conversion of adenine through hypoxanthine and xanthine to uric acid. These findings appeared to be supported by evidence which included the determination of urinary xanthine by a xanthine oxidase method based on that of Williams (1950).

Recent development of alternative methods of analysis for urinary purines has offered the opportunity of appraising these findings. Adenine, xanthine and hypoxanthine are excreted in quantities of only a few mg. per day, and only recently have methods developed by Weissmann, Bromberg and Gutman (1957) and Weissman and Gutman (1957) been available for their determination in less than a day's output of urine. Weissman's methods are largely chromatographic, and are well documented; they apply to the purines named and to several others. It was therefore considered of value to adapt his methods for repetitive determinations in groups of subjects available to us, so that information could be obtained on these matters and so that the suggestions of Kishimoto could be examined at the same time by an independent method.

It was also considered important, in view of the notorious difficulties associated with the clinical problems of schizophrenia, to attempt to minimize some of the more obvious experimental complexities. For this reason particular care was devoted to the selection of cases. A matching procedure was introduced which took into account the subject's age, sex, weight and nutrition.

METHODS

CLINICAL

The urinary purines were determined in a series of schizophrenic in-patients at the Bethlem Royal and the Maudsley Hospitals (case histories are appended), and in a matched series of normal subjects. The diagnosis was based on classical

data laid down by Bleuler, i.e. thought-disorder of a characteristic type with disturbance of volition and affect; in addition the presence of primary delusions was accepted as strongly suggestive of the illness. In the selection of schizophrenic patients an endeavour was made to obtain cases in the younger age groups with short histories and severe symptoms. The patients had all been labelled as suffering from unequivocal schizophrenia by physicians responsible for their care. As a further precaution two of the authors (R.C. and M.S.) made independent assessments of the clinical features at separate diagnostic interviews. A four-point rating scale was employed and only when both observers regarded each patient as falling within the two most abnormal categories was the patient accepted for investigation. Drug treatment was suspended a few days before testing except when such an interruption in therapy appeared likely to affect the patient's condition adversely.

Case 1

Mr. D.P., aged 30, was admitted to the Maudsley Hospital on 5 August, 1958.

There was no family history of mental aberration apart from a transient depressive illness which an older sister had developed at the age of 40.

The patient had developed normally and had made average progress at school. On leaving school he took up bricklaying for 4 years, served with the army in Egypt for 2 years and then worked as a lathe setter for 8 years until the onset of mental symptoms when he lost his job.

He had married happily at the age of 22 years; his wife was 3 years older than he; they had two healthy children.

The patient was described by his wife as having been a good mixer, not given to fits of depression. He was generally easy-going, quiet and methodical. His leisure interests had been mainly sporting.

Eighteen months before admission the patient began to interest himself in politics and simultaneously he neglected his other pursuits. He gave up all his usual activities and neglected his appearance. A few months later he suddenly became unreasonably anxious and complained of depression and insomnia. He was admitted to a mental hospital where he was given first E.C.T. without effect and then received 44 insulin comas, after which he seemed a little better. Eventually he took his own discharge.

Following his discharge he was unable to work; he felt depressed and complained of an inability to think clearly. He also told his wife that he could not control his thoughts and that he could hear voices inside his head repeating advice which he had been given in the past, e.g. "walk, watch and listen". He spent his time unoccupied at home before his admission.

On examination he was noted to be a burly, plethoric man in satisfactory physical health apart from a moderate hypertension. He was rather hostile to the examining doctor and made occasional odd grimaces and gestures. Several times he burst into tears for no reason. He seemed to have difficulty in expressing himself in speech and the meaning of his statements was often difficult to grasp. He stated that he had voices in his mind repeating instructions and that he felt as if he were thinking what others were thinking. He also complained that when addressed he seemed to visualize all subjects of conversation.

The administration of reserpine 3 mg. t.d.s. resulted in an improvement of his overactivity; it had, however, produced little effect on his mental state at the time of examination.

Case 2

Mr. K.W., aged 25, was admitted to the Maudsley Hospital on 24 June, 1958 on account of disturbed behaviour at home.

The family history was negative apart from psoriasis among some of his father's relatives.

The patient was born in Wales. His early development was normal, He was an average scholar and left school at the age of 16 years. He then took four short office jobs before entering the army for his National Service at the age of 18 years. In the army he frequently overstayed his leave and absconded several times. After making a suicidal attempt following the death of his father he was kept in detention for 6 months before his discharge from the service. During the following year he held three jobs briefly as an office filing clerk. At the age of 21 he developed a psoriatic rash over his scalp and forearms which subsequently spread all over his body.

Before his illness he had been cheerful and popular but sensitive to the opinions of others. He tended to be egocentric and he daydreamed to an unusual degree.

His illness began about 2 years before his admission, when he gave up attempts to find work. He claimed that if it had not been for his skin condition he would have found work though the rash was in reality not very severe. Four months before admission he attempted suicide with coal gas and his behaviour deteriorated subsequently. He became aggressive

towards his mother and refused to talk to her. During angry outbursts he smashed door panels and broke chains. He said that he could hear the voices of a lady who told him to pray for forgiveness and of a man who encouraged suicide. On several occasions he complained of having a woman inside him and of having his genitalia manipulated by a hypnotist. He often smiled and laughed to himself for hours and said it was difficult to think straight. Many times he exposed himself to his mother and masturbated.

On examination after admission he was noted to have a mild generalized psoriatic eruption. Mentally he was well behaved and smiled fatuously all the time. There was considerable flattening of affect as well as thought blocking and concreteness of thinking. Although his intelligence was average on formal testing he had difficulty in interpreting proverbs. He did not regard himself as ill and thought that anyone with psoriasis would behave as he had done. He was tested before drug-treatment was commenced.

Case 3

Mr. E.S., aged 35, was admitted to the Maudsley Hospital on 26 June, 1959.

There was no family history of mental disorder. The patient had developed normally and had made average progress at school. On leaving school at the age of 14 years he took unskilled jobs for 3 years until he was called up for the army in 1939. He served for 7 years and reached the rank of lance-corporal. After demobilization he worked as a cleaner, his longest spell at one job being 7 years.

He was married at the age of 26 years to a typist after an acquaintanceship of 6 months. The marriage was never harmonious; there were frequent quarrels over the upbringing of their two children. His wife was a perfectionist who insisted on unattainable standards of tidiness in the house.

Before the onset of his illness the patient was a rather quiet, moody, hard-working man with few friends. He was quick-tempered and careless over details. His main interests were in football pools and the cinema.

The illness was very gradual in onset. In 1953 he changed from day to night duty in order to observe his wife's handling of the children as he felt that she had been mismanaging them. In 1954 he left his job because of a quarrel with his supervisor and then became increasingly moody and subject to fits of temper. In 1956 he became sexually demanding and 6 months before his admission he complained of sabotage at his place of work and of an organized plot directed against him. He maintained that his workmate was mentally defective and that newspaper articles bore a special reference to him. Eventually he claimed that his workmates had been sent from the law courts to observe him and he became so distressed that it was necessary to summon the duly authorized officer.

On examination in hospital he was in good physical health. He was in a state of mild agitation and very suspicious of the staff and other patients. He demonstrated marked thought-disorder. Innocent remarks and gestures by other patients seemed to carry sinister meanings and he thought that certain television programmes referred to him. He was receiving chlorpromazine 150 mg. t.d.s. at the time of testing.

Case 4

Mr. N.O., aged 22 years, was admitted to the Maudsley Hospital on 2 May, 1959 from an observation ward.

There was no family history of mental illness. His father was an Italian waiter and his mother was of English stock. He was an only child.

His early development was unremarkable and he did well at school, both scholastically and socially. On leaving his grammar school he obtained a scholarship to an art school where he made good progress at first. His military service was deferred to enable him to complete his studies.

Before his illness he had been cheerful, stable and confident, with a few close friends of his own sex and many superficial acquaintances. He took a keen interest in art and philosophy.

Seven months before admission he became increasingly solitary, lost interest in painting and stayed away from art school. He daydreamed a lot at home and ate very little. He said that he could see a cross in the sky and he felt compelled to pray continuously and also to fast because, he maintained, Jesus Christ had done so. It was his stubborn refusal to eat which led to his admission to the observation ward.

On admission to hospital he was in good physical health. His psychomotor expression was restricted and his affective responses were flattened. His talk was rambling and imprecise and its meaning was uncertain. He spoke of "visual voices" which he defined as the voices of people whom he could see. He complained of feeling "out of tune" and stated that this feeling was related to "changing colours as the clouds pass over the sun . . . it takes the character of a moment". He was unable to interpret proverbs, tending to respond with another proverb. One month after admission insulin coma treatment was commenced but he refused further treatment after the first coma. He then began to talk of hearing the voices of a brother and sister in his thoughts. He was not receiving drugs at the time of testing.

Case 5

Mr. F.H., aged 17, was admitted to the Maudsley Hospital on 2 February, 1959 from a local general hospital to which he had been sent by his own doctor.

His mother, a German Jewess, had been in a mental hospital for some years for an illness which had been diagnosed as paranoid schizophrenia. The patient was reared by his father on account of his mother's illness. His father died early in 1958.

The patient's early development and school career were normal. On leaving school at the age of 16 years he began work as a shipping clerk, taking night-classes in shorthand and French.

He was described by his guardian as being normally a confident but sensitive youth with few close friends. He was shy with girls but at ease with older people. He tended to daydream.

During his last few months at school he had become increasingly shy and sensitive; his standard of school-work deteriorated and he obtained surprisingly low marks in the G.C.E. After beginning work he developed numerous hypochondriacal complaints and then accused his guardian's wife of organizing campaigns against him.

On examination he was a small, pale youth with cold blue hands. His talk was slow but normal in form. He complained of a fog over his mind and wondered whether he needed a brain operation as he could feel a tight band around his head which was making his head large and his body small. He also feared that he might be changing his sex. His mood was one of perplexity and apprehension. He attributed his morbid experiences to the efforts he had made to avoid accusing his aunt of starting campaigns against him. He was given 46 insulin comas without demonstrable effect. He was not under any drug treatment at the time of testing.

Case 6

Miss E.P., aged 23, was admitted to Bethlem Royal Hospital on 13 December, 1958.

The patient and her identical twin sister were adopted soon after birth by a well-to-do elderly couple and no information was available about the mental health of her true parents.

Her early development was slow and she was a difficult child with frequent temper tantrums and night terrors. At school she did poorly; during her final year she became a prefect for a time but was demoted the following term on account of her unkindness to younger girls.

Her periods were always painful and irregular. She had some homosexual inclinations in adolescence but never displayed heterosexual interests.

Prior to her illness she had been a shy, brusque, jealous girl lacking in self-confidence. She was affectionate towards her adoptive mother but had no close friends. She was a day-dreamer, very interested in ballet, music, plays and fairy tales. She was liable to sudden changes of mood and would sulk and cry for no apparent reason.

Her illness commenced insidiously. At the age of 18 years she started work at a secretarial college but left after 8 months: she found the pace too rapid for her and she was disturbed by homosexual feelings. After an attempt to take a domestic science course she tried several jobs, only to be dismissed each time for incompetence. She became hostile, self-absorbed and negativistic; she thought she might lose her reason. At the age of 20 years she entered a private hospital in Devon but had to be transferred to Bethlem Royal Hospital on 12 January, 1956. At that time she was unkempt, hostile, pouting, solitary and sullen, inclined to scream suddenly at the staff if she were frustrated. Her talk was disjointed with evidence of thought disorder; incongruity of affect was noted. Her attention and concentration were poor; there were no delusions or hallucinations. She was treated with reserpine and her behaviour slowly improved. She ate ravenously and put on much weight. In due course the dosage of the drug was reduced and she was well enough to be discharged 8 months after admission.

Two months later it was necessary to re-admit her as she had become agitated at home; she had accused her adoptive parents of influencing her mind and of working against her. She also stated that another lady aged 90 years was homosexual. Her clinical state was similar to that of her first admission except for her refusal to look at doctors and a determination to sit with her arms folded looking at the floor. She was treated with drugs at different times. Eighteen months after her admission she was sufficiently well to return home and take a typing job.

Five months later she again relapsed and was re-admitted. On this occasion her talk was less coherent than it had been but otherwise the abnormalities of her mental state were not substantially different from those which had been recorded previously at the time of testing.

Case 7

Mrs. E.M., aged 30, was admitted to the Maudsley Hospital on 23 February, 1959 from an observation ward to which she had been taken by the duly authorized officer on account of delusions, hallucinations and agitation at home.

She came from a poor farming family in Southern Ireland. There was no family history of mental illness. Her early development had been normal and she had suffered no significant illness or injury apart from a blow on the head at the age of 7 years, following which she was unconscious for some hours. She had been treated recently for anaemia. She made fair progress at school but left at the age of 12. She had won a competition as a beauty queen in Ireland. She married at the age of 20 years and two years later came to England where her husband obtained work in an iron foundry.

During the 7 years before her admission she had given birth to 7 sons and each pregnancy had been marred by severe vomiting in the first trimester. Following the last delivery 2 years before admission she was said by the nurses to be "hysterical". Her husband described her

as being normally somewhat moody, withdrawn and irritable; she was always considered "different" by her family. She had been a devout Roman Catholic until 3 years before her admission and had always been superstitious.

Shortly after her marriage while working as an usherette in a cinema she complained that men were following her but she produced no evidence to support her accusations. Her husband dated the onset of her illness to 5 years before admission when she expressed the persistent, irrational belief that her sister-in-law had dyed her child's hair. Three years before admission she ceased attendance at mass and expressed bizarre ideas about science. She complained bitterly about her living conditions. She became more preoccupied, withdrawn and suspicious and 2 months before admission began to scream at the window, apparently in answer to hallucinatory voices. She continued to deteriorate until her husband was obliged to call the duly authorized officer

On admission she was physically well but was withdrawn, suspicious and obviously hallucinated. Her talk was barely coherent and was broken by frequent silences. As she spoke she made praying gestures with her hands and at times she looked into one corner of the room and whispered. She expressed delusions that her body was changing, that she was being deprived of her thoughts, that her family had been killed and that her food was poisoned. As she discussed her symptoms she smiled and giggled to herself. Asked to interpret the proverb "A stitch in time saves nine" she laughed and said ". . . that means if you stitch your clothes some of them are more holy than God". She was examined in this condition.

Case 8

Miss B.C., aged 25, was transferred to the Maudsley Hospital on 10 July, 1959 from an observation ward to which she had been admitted one week earlier.

There was no family history of mental illness. The patient's development and school record had been unremarkable apart from attacks of bad temper as a child. From 17–22 years she had helped on her parents' poultry farm near Oxford. She then went to live for 18 months as the mistress of a man whom she had met on holiday at Torquay. At the age of 24 years she took a post as assistant matron at a boys' grammar school from which she was dismissed 2 months later (on 2 March, 1959) on grounds of unsuitability.

She had suffered no serious physical illness. Her previous personality had been marked by moodiness, short-lived depressive spells and a preference for her own company.

After her dismissal from the grammar school she took a train to London without a ticket saying that she was going to see her royal parents at Buckingham Palace and that she had been told to do this by voices. The station authorities summoned the duly authorized officer who transferred her to the observation ward.

On examination her extremities were noted to be cold but her physical health was otherwise satisfactory. Her speech was slow, vague in content and accompanied by incongruous smiles and laughter. She complained of feeling sad and strange and objected to seeing the name "Miss King" on the end of another patient's bed as she regarded this as an insult to her father.

She was given chlorpromazine 50 mg. t.d.s. with little effect on her mental state by the time she was examined.

Case 9

Mrs. H.S., aged 48, was admitted to the Maudsley Hospital on 27 July, 1959 from an observation ward.

She had been brought up in Wales, the seventh child of a family of nine, most of whom were illiterate. There was no family history of mental illness.

Her development was normal but she was a poor scholar. After leaving school at the age of 12 years she came to London as a domestic and had several short-lived jobs in the first few years.

She married at the age of 18 years in 1928, having known her husband for 2 years. He was only one year older than she but he remained unemployed until 1940 when he joined the army. The marriage was unhappy but they had 8 healthy children before separating in 1951. She had never wished her children to go to school and in consequence they had acquired little education. Two years before her admission the patient lived with another man who described her as being cheerful, argumentative and rather suspicious. She did her housework well but associated very little with other people as she felt they were always talking about her.

For about 25 years the patient had entertained the beliefs that she was Queen of England and that other people living above her in her home were trying to poison her. During the year before admission she expressed such notions more frequently. One day without warning she travelled from Wales to London where she was found by the police outside Buckingham Palace proclaiming herself to be Queen.

On admission to hospital she was in satisfactory physical health. Mentally she was co-operative and fairly sociable. She was correctly orientated and answered questions not related to her illness rationally and coherently. Her mood was cheerful but she became angry on seeing a photograph of the Queen, declaring that the person in the photograph was an imposter. She admitted to constant auditory hallucinations of confused talking and referred to "him up there", pointing to the ceiling. She stated that she owned the hospital and could move about as she liked. She thought her son might have changed his sex. Although stelazine 5 mg.

t.d.s. was prescribed she usually refused medication and her mental state was unchanged at the time of testing.

Case 10

Miss M.H., aged 28 years, was transferred on 27 July, 1959 to the Maudsley Hospital from an observation ward to which she had been admitted 9 days previously.

There was no family history of mental illness. Both parents were stable, intelligent people; the father was a journalist employed by Reuter in South Africa.

The patient was born in London and educated in residential convent schools as her family were often overseas. She matriculated at the age of 15 and undertook secretarial work until she was 21 years; she then studied for 6 months at the Sorbonne and worked as a translating secretary in South Africa for the next 7 years. She returned to England in 1957 and worked with the French Press Agency until her illness.

Before her illness she was regarded as a shy, nervous girl, a strict Roman Catholic who was attached to her family and who had few friends. She appeared to be without heterosexual interests.

She had suffered a previous illness 18 months before admission when she had been admitted to the Bethlem Royal Hospital where her affect was regarded as incongruous and her talk was noted to have been vague and incoherent. She had also displayed various bizarre, hypochondriacal delusions and believed there was something she could not describe growing in her rectum. She was treated with 21 insulin comas followed by a short course of chlorpromazine and seemed to make a full recovery. She had then gone to live with an aunt and she continued with her secretarial work.

Ten months after her discharge the patient asked her employer for a rise in salary. When this was refused she left her job and took a secretarial post elsewhere. Two weeks later she felt that the other people in the office were laughing at her and making suggestive remarks. On 15 June, 1959 she told her friends that she had appeared on television and that she was a medium. Three days later she set out to see her doctor but deliberately allowed herself to be accosted by an Indian; she felt that she had to permit this action as a gesture of atonement but became frightened and struck the man with an umbrella. She was found by the police and taken to an observation ward.

On admission she was in good physical health. Her talk was normal in flow and form but she stated her belief that her mind was being shared by somebody else and that people could read her thoughts at a distance. She also felt that the nurses were observing her for lesbian tendencies and that an outside agency was placing abusive words in her mind.

She was treated with stelazine 5 mg. t.d.s. and was improving at the time of testing.

The control series consisted of members of the staff of the Institute and the Joint Hospitals, matched with the patients in age, sex, weight and height. The degree of matching can be judged from Tables IX and X. The subjects were all ambulant during the experimental period and continued with their normal diet. In addition all caffeine-containing drinks, e.g. tea and coffee, were recorded in every case and note was taken of any special features of eating, drinking and behaviour during the test period. Since both staff and patients received some of their meals from the same kitchen the diets overlapped to some extent. From each subject an accurate 24-hour specimen of urine was collected in a large polythene bottle containing 10 ml. of chloroform as preservative. Nurses supervised collections made by patients. In a number of cases further 24-hour specimens were obtained from the same patient so that variations in urine output, their diet and changes in drug treatment were examined.

Determination of Urinary Purines

The methods of Weissmann *et al.* (1957) were applied, modified as detailed below for repetitive determinations with 1/10 of the daily output of urine. The methods involve removing the bulk of the uric acid from a fraction of a day's excretion, by keeping cold with acid; adsorption of the purines to a Dowex resin and their elution with ammonia; their precipitation with silver salts and separation by paper chromatography. On the paper, purine spots are identified in ultra-violet light, eluted individually, and measured spectrophotometrically.

The 24-hour specimens of urine were taken to pH 2 with 2 N-HCl (short range indicator paper), kept at 4° C. for 16 hours or longer, filtered through

fluted papers, and 1/10th of each specimen used for each determination. Determinations were carried out in duplicate, and in addition urine equivalent to a further 1/5th of the specimen was kept at −25° C. for future use.

(1) *Adsorption*

For adsorption, Dowex 50-H++, 8 per cent. cross-linked, mesh 20–50 or 50–100 was used in columns of length 21 cm. and diameter 2 cm. ,requiring about 50 ml. of resin. Columns were prepared by washing with (*a*) 50 ml. 2N-NaOH, (*b*) water until neutral, (*c*) 100 ml. 2N-HCl and (*d*) water again until neutral to short range indicator paper. The cycle (*a*) to (*d*) was repeated. The washings were discarded.

As the quantity and particle-size of the resin differed from that previously employed by Weissmann *et al.* (1957), a trial experiment was necessary to determine volume of 1 N-NH_4-OH for complete elution. For this purpose 1/10th of a 24-hour urine specimen was dripped slowly for 1 hour through a column of the Dowex 50-H^+ resin in 50–100 mesh size and the column washed with 250 ml. water. (On some occasions the same resin in 20–50 mesh size was employed: see below.) Fractions of 2·5 ml. were collected from the column by eluting with 200 ml. 1 N-NH_4-OH, followed by 50 ml. water. Optical density of the eluates at 260 mμ was determined after suitable dilution with water and acidification, with 1 N-HCl. A large increase in optical density occurred in specimens collected after about 100 ml. NH_4OH had passed, and was maintained until about 140 ml. were collected (Fig. 1); it was about coincident with the appearance of a yellow colour from the urine. On this basis the standard procedure adopted was to pass the material from 1/10th of a 24-hour collection of urine through the column described, at the rate of 150 ml./hour, followed by 200 ml. of water. The effluents were discarded and the purines eluted with 200 ml. of N-NH_4OH followed by 50 ml. of water; to ensure that minor constituents were not lost, these two effluents were combined and used in the following stage.

(2) *Silver Salt Precipitation*

The samples after elution were reduced in volume to 3 ml. or less with a rotary evaporator at 20 mm. Hg in a bath at 55°, and pipetted into tubes previously calibrated at 10 and 20 ml. The solutions were taken to pH 2 with N-H_2SO_4 and 2 ml. N-$AgNO_3$ added, the final volume being adjusted to 10 ml. with water. Tubes were left for 24 hours at room temperature in the dark and for a further 48 hours at 0°. They were then centrifuged (1,500 g.) and the supernatant removed. The silver precipitates were carefully washed with 5 ml. portions of water until the supernatant became colourless (2 or 3 washings). The purine-silver complexes were decomposed by adding 20 ml. hot 0·05 N-HCl and heating 5 minutes on a boiling water bath. The tubes were centrifuged as before and the supernatant retained. The precipitates were washed with further quantities of 10 ml. 0·05 N-HCl. The combined washings or aliquots from them were concentrated to dryness in a round-bottomed flask in vacuum, and any remainder not immediately used was stored at −25°.

(3) *Paper Chromatography*

The procedure recommended by Weissmann *et al.* (1957) was adopted for two-dimensional chromatography, the concentrate being applied by 3 successive additions of 20 μl. with calibrated capillary pipettes. The first run was with a

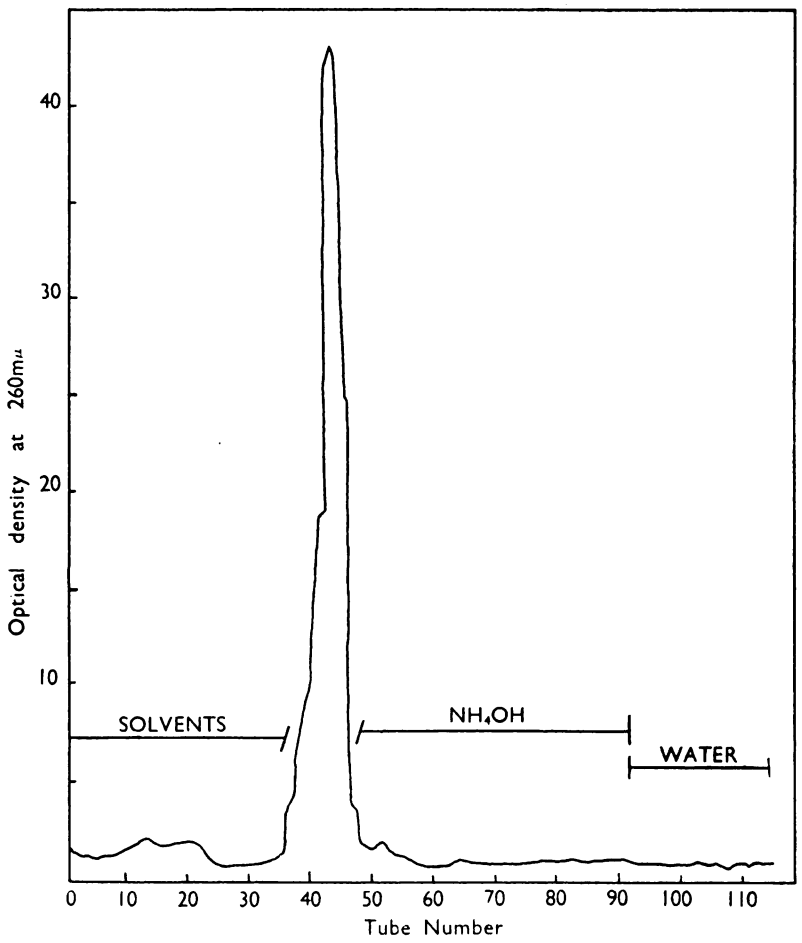

FIG. 1.—Purine analysis: Elution from a column of Dowex −50 H+. Urine, water, 200 ml. N-NH_4 and 50 ml. water were passed through the column as described in the text. The effluents were collected in 2·5 ml. portions in separate tubes, and the optical densities of these samples determined directly or after appropriate dilution.

mixture of n-butyl alcohol (Hopkins and Williams, chromatographic grade) and 0·6 N-NH_4OH (6:1, v/v), for approximately 48 hours. After drying for 24 hours in a stream of air at room temperature the papers were run in a direction at right angles to the previous run, with a freshly prepared mixture of n-butyl alcohol, formic acid and water (77; 11:12 v/v). Spots were located by ultra-violet light with a Hanovia lamp with its main emission at 253·7 mμ. For identification, other sheets were run in the same tanks with known quantities of authentic specimens of adenine, guanine, hypoxanthine and xanthine. Later there was found to be sufficient internal evidence for identification of spots with urine specimens alone (Fig. 2). Adenine was always the fast-moving spot in the butanol-ammonia solvent. Guanine which only occurred in trace amounts and the 1-methyl and 7-methyl guanines which run as one spot, exhibited a strong blue fluorescence when papers still moist from the second solvent system were irradiated by ultra-violet light. Hypoxanthine ran nearly parallel with the latter spot in this solvent but more rapidly in butanol-formic acid (Fig. 2).

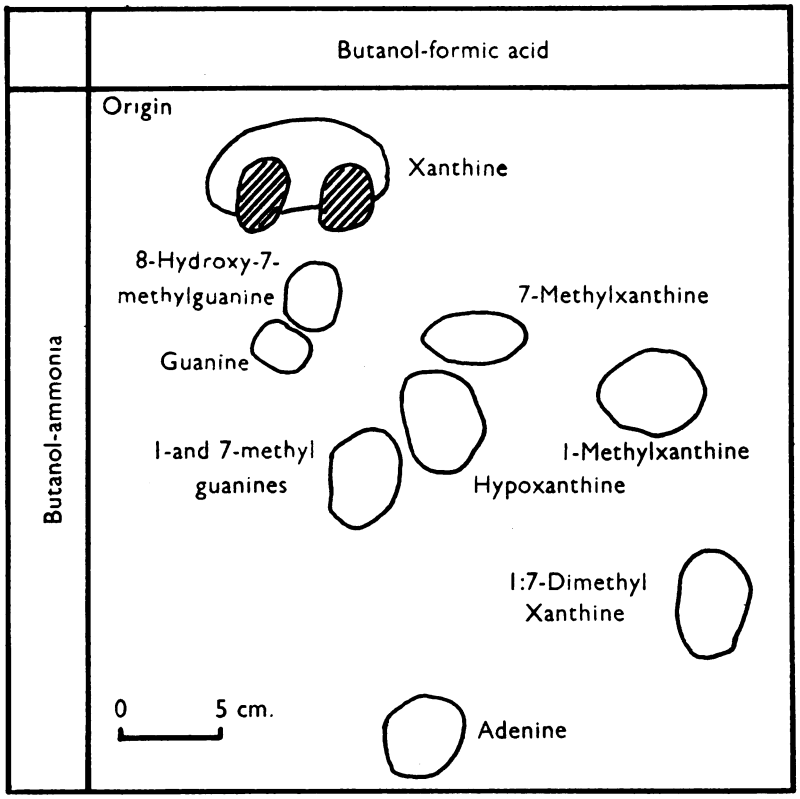

FIG. 2.—Purine-like materials obtained using the standard procedures described under Methods. Materials underlined were those employed as markers for the identification and location of other purines. 7-Methylxanthine, 1-methylxanthine and paraxanthine were from dietary sources. Certain fluorescent materials associated with xanthine are indicated with hatching. The origin is marked by (O) in the upper left-hand corner.

Xanthine as noted remained nearer the origin and was always associated with two characteristic fluorescent spots. Other spots were located in relation to those already mentioned.

(4) *Elution Technique*

Spots located by ultra-violet light were cut out, and neighbouring areas of the chromatogram of the same size but free from obvious absorption and fluorescence were also taken to act as controls. The papers were cut into pieces 0·5 cm. square or smaller, placed into tubes with 3·5 or 7 ml. water, and macerated. After 15 minutes the suspension was filtered by suction through a small glass funnel through the stem of which was placed a small pointed glass rod, with flattened end above which carried a filter paper about 6 mm. in diameter. The conditions of elution were based on trials in which 25 μg of hypoxanthine, xanthine, adenine and guanine were extracted with different volumes of solvent for times of 15 minutes to 16 hours, with results shown in Tables I and II. Elution with water proved to be rapid and complete (Table I). In initial trials the four bases were eluted with 3·5 ml. of water, with results (Table I) indicating that 15 minutes was adequate for elution. It will be noted from Table II that the extracts from control areas of paper, obtained under

TABLE I

Recovery of Purines from Chromatographic Procedures

Compound Placed on Paper (μg)		Yield on Elution with Water for: 15 Minutes 1st 3·5 ml. (μg)	15 Minutes 2nd 3·5 ml. (μg)	15 Minutes Total Per cent.	16 Hours 1st 3·5 ml. (μg)	16 Hours 2nd 3·5 ml. (μg)	16 Hours Total Per cent.
Hypoxanthine	25·1	23·2	1·1	97	22·6	1·3	95
Xanthine	20	18·3	1·2	97·5	16·8	1·7	92·5
Adenine	30	27·5	1·6	97	27·5	2·0	89
Guanine	22	19·5	1·4	95	17·5	2·0	89

The purines were applied to a 35×38 cm. sheet of Whatman 3 mm. paper, which was dried and run in butanol-ammonia.

Spots were located and cut out, and control areas of paper taken, as described in the text (see also Table II). A third elution yielded no further absorbing material.

TABLE II

Recovery of Hypoxanthine Standards from Chromatographic Procedures

Hypoxanthine Added (μg)	Elution Volume (ml.)	Optical Density of Eluates: Standard	Optical Density of Eluates: Control	Hypoxanthine Recovered: μg	Hypoxanthine Recovered: Per cent.
25·1	3·5	0·564	0·045	23·2	92·5
50·2	3·5	1·064	0·045	45·5	91
25·1	3·5*	0·565	0·060	22·6	90·5
—	3·5†	0·087	0·057	1·3	95·0
25·1	5·0	0·399	0·027	24·0	95·5
50·2	5·0	0·793	0·027	49·0	97·5
25·1	7·0	0·295	0·021	24·6	98·0
50·2	7·0	0·556	0·021	49·0	97·5

Specimens placed on sheets of Whatman 3 mm. paper with the same capillary pipette, volume 20 μl., and delivery 25·1±0·2 (3) μg hypoxanthine (measured directly in 3·5 ml. water). Results are obtained after subtracting values with solutions from control areas of paper. Each value is the mean of duplicate readings agreeing within 5 per cent.

* Elution for 16 hours; others employed 15 minutes elution.

† A second 16-hour elution of sample and control; the per cent. recovery quoted refers to the sum of material from the first and second elutions.

different conditions, did not give the same absorption readings and it was thus necessary to take separate control areas for the different spots. The control values however remained less than 1/10th of those given by the purine-containing areas. In actual determinations in the urine extracts, control values were greater; thus in the experiments of Table V they were approximately 1/5th of values from the purine-rich areas. Elution for longer periods, or repeated extraction, gave increased elution in control papers from areas without purines and is thus undesirable. Results indicate that for spots expected to contain some 50 μg. of purine, a volume of 7·0 ml. gave recovery of 97 per cent. or more. These recovery values were obtained with hypoxanthine, xanthine, 1-methyl, 7-methyl and 1:7 dimethylxanthine. For spots of some 25 μg., elution in 3·5 ml. is recommended (Tables II and III).

(5) *Spectrophotometric Measurement and Calculation*

The eluates were buffered at pH 2·1 by adding 35 μl. of a solution 0·5 M in HBO_3, M in Na_2HPO_4 and 2·4 N in HCl, and absorption determined at chosen wavelengths with a Hilger Uvispek instrument. In initial experiments,

the absorption spectra of the materials eluted from urine were measured at a range of wavelengths and at other pH values in order to establish their identity. The findings agreed with those of Weissmann *et al.* (1957) and earlier workers, and accordingly the previously recorded extinction maxima and extinction coefficients were adopted in the observations and calculations of the present study. The values employed are quoted in Table III. The sample applied to the

TABLE III

Photometric Data Employed

Spot	E Maximum (mg./100 ml.)	Absorption Maximum (mμ)
*Hypoxanthine	0·78	248·5
Xanthine	0·64	266
*1-methylguanine with 7-methylguanine	0·62	250
*Adenine	0·93	262
8-hydroxy-7-methylguanine	0·88	248
*Guanine	0·69	248
1-methylxanthine	0·70	266
7-methylxanthine	0·60	268
Paraxanthine	0·53	268

The data is derived from sources indicated by Weissmann *et al.* (1957).

* Areas of paper bearing these compounds were eluted in 3·5 ml. water, and the others in 7 ml.

paper is 60 μl. of 800 μl., derived from 1/10th of the 24-hour output of purine; accordingly:

$$\text{Excretion (mg./day)} = \frac{\text{density read}}{E_{max}} \times \frac{800}{6} \times \frac{3 \cdot 5}{100}$$

when readings are made with 1 cm. cells, of material eluted in 3·5 ml. (E referring to mg./100 ml.).

(6) *Recovery of Purines Added to Urine: Repetitive Determinations*

Recovery experiments were carried out with guanine, adenine, hypoxanthine and xanthine, added to urine specimens in concentrations comparable with those normally excreted. Application of the full procedure described above showed mean recoveries of hypoxanthine of 97, xanthine of 64, adenine of 79 and guanine of 78 per cent. (Table IV). These were close to recovery values obtained by Weissmann *et al.* (1957) from an artificial mixture containing some constituents of urine.

Consistency of results on repetitive determinations was appraised under the following circumstances: (*a*) between duplicate determinations carried out with all specimens on the same occasion; (*b*) between determinations on the same specimens before and after storage; and (*c*) between different specimens from the same subject voided at different times.

(*a*) An example of determinations carried out with specimens from one pair of subjects is quoted in Table V, indicating the degree of agreement between the duplicate determinations of each purine normally carried out with all specimens. In the further example of Table VI, four determinations of each purine were carried out and the variation among the results is expressed as

TABLE IV

Recovery of Purines from Urine Specimens

	(*a*) Purine Output (mg./24 hour)	(*b*) Purine Added (mg./24 hour specimen)	(*c*) Purine Found (mg./24 hour specimen)	Difference (*c-a*)	Recovery (Percentage $\frac{c-a}{b} \times 100$)
Hypoxanthine	6·0	9·0	14·0	8·4	93·5
	5·0	8·4	13·2	8·2	98
	5·4	10·0	14·6	9·2	98
Xanthine ..	5·5	6·8	9·8	4·3	63
	4·8	5·0	7·75	2·95	68·5
	4·2	5·0	7·2	3·0	60
Adenine ..	0·9	3·1	3·6	2·7	87
	1·1	3·0	3·2	2·1	70
	1·0	3·0	3·5	2·4	80
Guanine ..	0·4	2·0	1·9	1·5	75
	0·3	2·1	2·1	1·8	84
	0·2	0·5	0·6	0·4	75

Specimens of purines listed were added together to urine in concentrations approximating to the expected daily excretion. This was carried out with three distinct urine specimens and the results compared to those from similar volumes of urine not receiving the additions.

TABLE V

Determinations Carried Out with Urine Specimens from a Healthy and Mentally-Ill Subject

Measurement	Healthy Male (P.W.)	Patient (F.H.)
Age (years)	18	17
Weight (kg.)	60·5	50·5
Urine volume (ml.)	1,408	805
Urinary creatinine (g./24 hours)	1·21	1·15
Daily creatinine (mg./kg.)	20	23·5
Purine excretion (mg./24 hours):		
Hypoxanthine	9·4, 8·7	6·75, 6·0
Xanthine	1·2, 2·3	3·4, 4·5
1- and 7-methylguanines	3·1, 3·2	4·54, 4·15
Adenine	1·2, 1·04	1·63, 1·62
8-hydroxy-7-methylguanine	0·43, 0·45	0·99, 0·99
Guanine	0·2	0·2

Neither subject was receiving drugs. Specimens were voided during the same period of 24 hours, and handled together throughout all determinations (e.g. during adsorption, chromatography and elution).

a standard deviation. After determinations had been carried out in duplicate with 20 urine specimens, their consistency was appraised as shown in Table VII. These results, and also those of Table VI, show greatest consistency in the determination of hypoxanthine, followed by xanthine, adenine and 7-methylguanine; values for 8-hydroxyguanine were markedly more variable.

(*b*) Volumes of urine specimens sufficient for duplicate determination were stored at −18° C., and purine estimations performed after three months

TABLE VI

Repetitive Determination of Purines from Two Subjects

Determination	Healthy Male (J.B.)	Patient (Mr. O)
Age (years)	22	22
Weight (kg.)	50	50
Urine volume (ml.)	1,630	1,167
Daily creatinine (mg./kg.)	31·6	21
Hypoxanthine	5·5	4·7
	5·45	4·8
	5·75	3·9
	5·95	4·36
Mean±S.D.	5·7±0·23	4·44±0·4
Xanthine	2·0	3·95
	2·2	3·4
	2·54	2·66
	2·7	3·3
Mean±S.D.	2·35±0·4	3·33±0·53
7-methylguanine	3·55	2·95
	3·3	3·15
	3·3	2·85
	3·9	2·34
Mean±S.D.	3·46±0·32	2·82±0·42
Adenine	1·2	0·91
	1·3	0·85
	1·4	0·54
	1·6	0·70
Mean±S.D.	1·37±0·17	0·75±0·16
8-hydroxy-7-methylguanine	0·2	0·73
	0·1	0·83
	0·2	0·3
	0·3	0·72
Mean±S.D.	0·20±0·08	0·64±0·23
Guanine	>0·2	>0·2

Purine determinations were carried out in two groups, the first as described in Table V and yielding the first two values in each group of four below, and the second group from distinct aliquots run on a subsequent occasion. Other details as in Table V.

Neither subject was receiving drugs.

TABLE VII

Consistency of Purine Determinations

Determination	Difference Between Duplicates (Per cent. of mean value±S.D.)
Hypoxanthine	4·5±3
Xanthine	12 ±7
1- and 7-methylguanines	13 ±7
Adenine	11 ±8
8-hydroxy-7-methylguanine	25 ±5·5

Purines analyses were carried out with 20 urine specimens, 10 from healthy subjects (5 males and 5 females) and 10 from patients (5 males and 5 females) yielding duplicate values of which Table V quotes examples. The differences between each pair of duplicates was expressed as a percentage of their mean value, the percentages averaged are here quoted with their standard deviations. Guanine excretion was too small for comparable appraisal.

TABLE VIII

Purines in Urine Specimens Kept For or Voided After Intervals of Some Months

Experiment	1		2		3			4		
Subject	C.P. (male, healthy)		E.S. (male patient)		R.C. (male, healthy)			E.M. (female, patient)		
Determination	(a)	(b)	(a)	(b)	(a)	(b)	(c)	(a)	(b)	(c)
Circumstances of storage or collection of urine	—	Same specimens after 7 months' storage	—	Same specimens after 7 months' storage	With drug	Fresh specimen 1 month later	Fresh specimen 7 months after (a); no drug	—	Same specimen after 3 months' storage	Fresh specimen 4 months after (a)
Hypoxanthine	5·0	4·6	7·8	6·5	4·8	6·9	5·2	3·8	3·45	4·0
Xanthine	1·1	1·2	2·0	2·0	2·1	4·3	4·0	3·7	3·4	3·9
1- and 7-methylguanines	2·4	2·1	8·9	7·0	3·85	5·2	4·5	2·1	3·0	3·5
Adenine	1·2	1·0	1·4	1·1	1·8	2·35	1·5	0·4	0·7	0·93
8-hydroxy-7-methylguanine	Not determined	0·75	1·0	0·8	0·6	1·32	0·2	Not determined	Not determined	0·97
Guanine	0·4	<0·2	<0·2	<0·2	<0·2	<0·2	<0·2	<0·2	<0·2	0·2

Purine excretions are expressed as mg. excreted in 24 hours. Specimens stored were kept at −25° C. for periods specified in the Table. For experiments 2a, 2b and 3a the subjects had been receiving chlorpromazine in daily doses of 250–450 mg.

and seven months. Change even after seven months was not large, but probably implies a small loss in most constituents on storage (Table VIII).

(*c*) Appreciable differences were found in some purines when specimens were obtained from the same individuals at intervals of some months, and analysed without storage (Table VIII). Weissmann *et al.* (1957) also reported such differences.

Other Determinations

The volume and pH of the urine were noted. Creatinine was determined in 0·5 ml. specimens by adding 20 ml. sat. aqueous picric acid, 1·5 ml. 10 per cent. w/v NaOH, allowing to stand for 15 minutes, and adding water to 100 ml. After 10 minutes optical density was read at 520 mμ. On keeping at 0° the urine specimens which had been collected over chloroform, their creatinine content was found to fall after some months, though creatinine standards similarly stored were stable. This occurred to similar degrees in acidified urines and in others left without adjustment of pH. A 24-hour specimen originally containing 1·62 g. creatinine lost 5 per cent. of its content in 47 days and 16 per cent. in 82 days.

The creatinine determinations were carried out and are recorded because it was considered desirable to include the estimation of a well-investigated urinary constituent in the present study. Urinary creatinine values can give an indication of gross deficiencies in the completeness of the collection of the day's urine output. Such incompleteness was not encountered in the present study, for the collections were carefully made. Creatinine excretion is reported in Tables V, VI, IX and X in terms of body weight, and it will be noted that the values imply a fairly wide range of "normal" variation in creatinine excretion: in males, from 21 to 32, mean 24·9$\pm$4·0 (5) mg./kg., and in females, from 12 to 25, mean 18·8$\pm$4·2 (7) mg./kg. The value of 20 mg./kg. described by some writers as normal is recognized as susceptible to large variation (Folin, 1905; Hunter, 1928; Beard, 1943; Vestergaad and Leverett, 1958).

Results

1. *Comparison of Schizophrenic and Control Subjects*

The purine excretion figures for the male and female groups are shown in Tables IX and X.

When the results for hypoxanthine are studied it is noted that in 6 out of the 10 pairs the patient's excretion of hypoxanthine exceeded that of the control. Application of student's t test to the data indicated that the difference fell well short of significance. This appraisal was not altered when body weight is taken into account (Fig. 3). Similarly when figures for xanthine are considered it is found that in 7 out of the 10 pairs the patients' excretion was higher than that of the control, but again the difference fails to approach significance. When the male and female groups are considered singly with respect to the substances the significances of the differences are not increased. When the output of the other purines is reviewed the schizophrenics and controls seem to be a homogeneous group.

Since there appeared to be no striking group differences between the patients and the controls, individual variations from the group means were examined in terms of particular purines and specific clinical features—severity of illness, duration of illness, and form and course of these illnesses. No relationship was apparent. It is, for example, evident from Tables IX and X

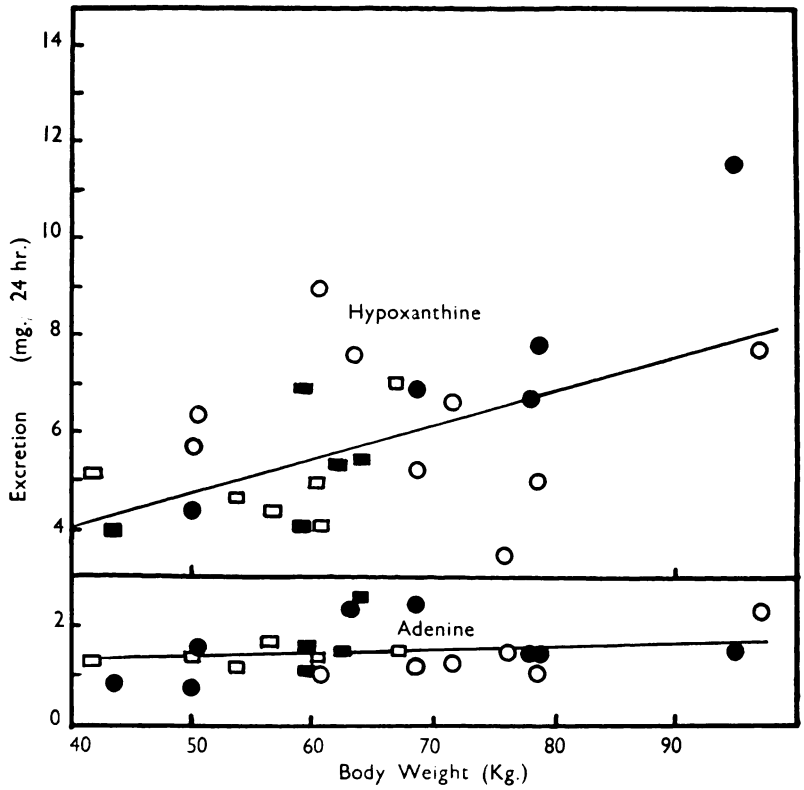

FIG. 3.—Daily excretion of hypoxanthine and adenine by 14 men and 14 women. Half of each sex were schizophrenic and half were normal subjects who were approximately matched in age and weight. The lines show the calculated regression coefficients.

that hypoxanthine excretion was particularly high in patients 1 and 3. References to these patients' case histories, however, failed to indicate that these two men differed clinically from the remainder of the group or that they displayed particular clinical features in common. Again, xanthine output was low in patients 2 and 3; 1-methyl and 7-methylguanine output was high in patients 3 and 8. Hypoxanthine and 7-methylguanine excretion was raised in patient 1 but there was no discernible connection between these variations and the characteristics of the illnesses from which the patients suffered. Furthermore, it will be seen from Tables IX and X that the control subjects showed deviations of similar frequency and magnitude to those of the patients. On this evidence it may be suggested that marked deviations in output of the purines were without clinical significance. Purine excretion was also unrelated to the particular treatment of drugs which had been administered to the patients in periods prior to those in which urine specimens were collected for the present studies.

2. *Variation with Body Weight*

Although variation in the excretion of endogenous purines in the present experiments was considerably less than in those from dietary sources (see below), there were sufficient differences within the groups of 28 males and females

TABLE IX

Purine Excretion in Male Subjects

Pair	Person	Age (years)	Weight (kg.)	Urinary Volume (ml./24 hours)	Creatinine (mg./kg.)	Purine Excretion (mg./24 hours)								
						Hypo-xanthine	Xan-thine	1-methyl and 7-methyl-guanine	Adenine	8-hydroxy-7-methyl-guanine	Guanine	Para-xanthine	1-methyl-xanthine	7-methyl-xanthine
1 ..	P*	30	95	1,540	11·5	11·6	4·2	2·5	1·55	1·45	—	6·6	23·4	17·2
1 ..	C	37	97	3,380	13·2	7·7	4·9	2·8	2·34	1·47	—	13·6	19·0	20·3
2 ..	W*	23	78	1,080	17·5	6·7	1·6	4·6	1·46	—	0·32	3·7	5·2	17·3
2 ..	C	32	76	2,600	20·6	3·5	2·9	2·1	1·5	0·91	—	9·4	10·2	13·9
3 ..	S*	35	78·5	2,250	13·9	7·8	2·0	8·9	1·4	1·0	—	5·6	17·0	8·9
3 ..	C	29	78·4	2,290	23·2	5·0	1·1	2·4	1·2	—	<0·2	3·0	3·0	2·4
4 ..	O*	22	50	1,167	21	4·4	3·3	2·8	0·75	0·64	<0·2	—	—	—
4 ..	C	22	50	1,630	31·6	5·7	2·3	3·5	1·4	0·2	<0·2	—	—	—
5 ..	H*	17	50·5	8·5	23·5	6·4	3·9	1·6	1·6	1·0	<0·2	—	—	—
5 ..	C	18	60·5	1,406	20	9·0	1·8	1·1	1·1	0·44	0·2	—	—	—

* Schizophrenic. C, control subject from whom samples were obtained at the same time, and analysed together with the corresponding patient. C was chosen as similar as possible to the patient in age and weight.

—: Not determined.

TABLE X

Purine Excretion in Female Subjects

Pair	Person	Age (years)	Weight (kg.)	Urinary Volume (ml./24 hours)	Creatinine (mg./kg.)	Purine Excretion (mg./24 hours)								
						Hypo-xanthine	Xan-thine	1-methyl and 7-methyl-guanine	Adenine	8-hydroxy-7-methyl-guanine	Guanine	Para-xanthine	1-methyl-xanthine	7-methyl-xanthine
6 ..	P*	23	64	1,590	19·7	5·4	6·7	7·1	2·6	2·0	0·4	26·6	40	4·3
6 ..	C	25	53·5	1,340	19·7	4·6	4·9	3·0	1·2	1·1	0·4	12·4	12·5	1·6
7 ..	M*	30	43·5	1,040	18	4·0	3·9	3·5	0·95	0·97	<0·2	—	—	—
7 ..	C	29	60·3	1,070	15	4·1	2·95	4·0	1·34	<0·2	<0·2	—	—	—
8 ..	C*	25	59·4	2,000	18·5	6·9	5·6	2·94	1·6	—	0·4	—	—	—
8 ..	C	27	41·5	1,700	21·6	5·1	5·0	2·5	1·3	—	0·4	—	—	—
9 ..	S*	48	59·5	1,342	23	4·0	4·0	3·2	1·1	1·2	0·4	—	—	—
9 ..	C	48	67·0	1,670	15	7·0	8·4	6·15	1·5	2·0	<0·2	—	—	—
10 ..	H*	28	62·0	1,160	12	5·4	5·0	5·4	1·5	0·43	<0·2	—	—	—
10 ..	C	28	56·5	1,080	20	4·4	3·4	5·0	1·64	0·3	<0·2	—	—	—

* Schizophrenic. C, control subject from whom samples were obtained at the same time and analysed together with the corresponding patient. C was chosen as similar as possible to the patient. in age and weight

—: Not determined.

(Tables IV, V, VI, IX and X) to merit examining whether the variation was correlated with body weight.

The rate of *hypoxanthine* excretion is plotted against body weight in Figure 3 and shows considerable scatter, but a tendency to increase with increasing body weight. Two men over 90 kg. in weight gave the highest excretion rates, whilst the females who tended to be of lower weight than the males, are grouped almost exclusively in the lower half of the curve (Fig. 3). The calculated regression coefficient was 0·07, corresponding to a correlation coefficient of 0·28 and a P value of 0·05 to 0·1.

Plotted in a similar manner, the daily *adenine* excretion proved to be almost independent of body weight. Values for 1- and 7-methylguanines and for *xanthine* were too variable for a definite conclusion to be drawn. *Guanine* excretion was too small for comparable appraisal.

3. *Age and Sex*

Comparison of the male subjects (patients and healthy controls), with the corresponding female series (Tables IX and X) indicated differences between the sexes in the excretion of certain purines. Most noticeable in this respect was the smaller excretion of hypoxanthine in women, although since their weight was lower than the weight of the men, a certain difference would be expected for this reason alone. Also, the earlier analyses included more men and seasonal factors could be involved. In order to examine more closely any dependence of excretion on sex, specimens from normal men and women who were matched in age and weight were collected and analysed together (Table IX). In these experiments also, output of most purines was greater in the men. This applied to hypoxanthine.

The majority of the individuals examined were under 30 years of age. The highest value for hypoxanthine in men are in those aged 30 or over (Table IX) although this was not the case in the women. Excretion of other purines did not appear to be closely correlated with age.

4. *Dietary and Endogenous Purines*

Much of the purine excretion from subjects drinking ordinary quantities of tea, coffee or cocoa consists of caffeine, theophylline, theobromine or their metabolites, that is, mainly methylated xanthine and uric acid derivatives (Christman, 1952; Sollmann, 1957). The present methods are fully adequate for the determination of these metabolites as well as the endogenous purines, and such determinations were made in a proportion of the subjects under investigation, because abnormal metabolism of a dietary purine would be relevant to the present study. Values are included in Tables IX and X. These show 1-methylxanthine to be excreted at rates ranging from 3 to 40 mg./day, 7-methylxanthine at 2–20 mg./day and paraxanthine at 3 to 14 mg./day. These widely-spaced values were found to be approximately correlated with wide differences which were noted in the intake of tea or coffee. Differences were not observed between normal subjects and patients, but as can be judged by the values quoted, large differences only would have been significant. The large range of values observed in the excretion of the dietary purines, contrasts with the much narrower range observed in adenine, xanthine, hypoxanthine, guanine and methylated guanines (Tables IX and X), which were the main subject of the present study.

DISCUSSION

Excretion in Normal Subjects

The methods for determining urinary purines devised by Weissmann *et al.* (1957) were found readily adaptable to the present study. Before appraising findings in mentally-ill subjects the following characteristics of the methods employed and of purine excretion in normal subjects, are to be noted.

Recovery experiments: dietary factors. The division of the purines estimated into two groups, one greatly dependent on the intake of beverages containing caffeine and related compounds, and the other independent of these, is supported. Data on the first category of compounds, quoted in Tables IX and X, lie within a rather wide range of values quoted by Weissmann *et al.* (1957) and earlier observers. The other category of urinary purines, which may be termed endogenous, has been the subject of careful recovery experiments (Tables IV to VII), for recovery experiments of this type do not appear to have been carried out previously. Our results are actually similar to those of the recovery experiments made by Weissmann *et al.* (1957) from an "artificial urine" prepared from a limited number of pure substances contained in urine. Such a mixture must omit many more compounds than it includes, and thus recovery from urine itself is considered important. Recovery in the present experiments was excellent or fairly good in the cases of hypoxanthine, adenine and guanine, but of some 64 per cent. in the case of xanthine. The range of values found for excretion of "endogenous" purines from the present subjects usually overlapped with those reported by Weissmann *et al.* (1957); individual compounds are considered below. It is however a notable conclusion of the present study that excretion can depend markedly on bodily characteristics of the people examined, especially on their body weight and sex. That these characteristics have not been recorded in sufficient detail in earlier studies, limits the comparisons which can be made between the present and earlier findings, but the following conclusions can be drawn.

Hypoxanthine. The mean daily excretion of hypoxanthine by the present subjects of some 6 mg./day is appreciably lower than that found by Weissmann *et al.* (1957), of 9·7 (range: 7·4–12·5) mg./day. Recovery of added hypoxanthine in the present experiments was excellent, and the divergence from Weissmann's results must be attributed to a real difference between the two groups of subjects. Here the results of Figure 3 are important, for they show a marked dependence of hypoxanthine excretion on body weight, such that within the range of body weight examined a 2-fold difference can be attributed to this factor.

The other important characteristic now found to condition hypoxanthine is sex, as is apparent from Tables IX, X, XI and from Figure 3. The mean values from the investigation as a whole were: from men 6·78 and from women 5·09 mg./hour, that from the men being 1·33 times that from the women. This is similar to the well-established dependence of creatinine excretion on sex (Beard, 1943; Hunter, 1928). Indeed the mean value for creatinine excretion, per kg. body weight per day in the men now studied, was found to be 1·33 times that of the women.

Xanthine. The mean value for the daily excretion of xanthine by the present subjects, of 3·83 mg., is also about two-thirds of that found by Weissmann *et al.* (1957), which was 6·1 mg.; recovery of xanthine in the two studies was about the same. Figure 4 shows that we find large individual variations in xanthine among different people, though Table VIII indicates relatively constant values

TABLE XI

Purine Excreted in Male and Female Subjects Examined Together

Experiment number	1		2		3	
Subject (sex)	J.S. (male)	H.R. (female)	R.R. (male)	E.P. (female)	R.H.C. (male)	— (female)
Age (years)	27	29	40	43	30	35
Weight (kg.)	71·5	60·5	63·5	67	68·5	66·5
Urinary volume (ml.)	3,040	890	1,200	1,420	1,743	1.083
Daily creatinine (mg./kg.)	21	17	21	18·3	28	25
Purines (mg./24 hours):						
Hypoxanthine	6·6	4·6	7·6	3·55	5·2	5·2
Xanthine	3·7	3·25	3·7	3·3	4·0	2·0
7-methylguanine	3·2	1·7	3·92	4·0	4·5	2·2
Adenine	1·23	1·01	2·45	1·25	1·5	1·2
8-hydroxy-7-methylguanine	0·2	0·12	0·3	0·73	1·72	0·7
Guanine	<0·1	<0·1	0·1	0·2	<0·1	<0·1

Each experiment with a pair of subjects (one male, one female matched approximately in age and weight) was carried out as described in Table V.

in a given person examined on different occasions. No correlation with body weight is apparent in all or in any one of the four categories of subjects illustrated in Figure 4. There is also no consistent difference between the sexes in xanthine excretion; although Tables IX and X would suggest greater excretion in women than in men, this trend is not borne out by the determinations of Table XI which were made on samples which were collected and analysed together. Here the men give the higher xanthine values. We place greater reliance on this latter result as the experiment was designed with sex as the main variable; the collections and chemical analyses of Table IX were in most cases carried out a few months before those of Table X.

Other compounds. Excretion of adenine by the present people, of 1·45 mg./day, is close to the value of 1·4 mg. found by Weissmann *et al.* (1957). It has not been found dependent on the weight or age of the subjects, but all the values for men in Table XI are greater than those for women. Guanine is excreted in markedly lower quantities, of some 0·2 mg./day, which the present methods did not evaluate. Nevertheless, the recovery experiments of Table IV show that any appreciable increase in guanine excretion would have been detected; none was observed.

Excretion in Schizophrenia

The point at which direct comparison can be made between the present results and those of Kishimoto (1958) is in xanthine excretion. Here the Japanese workers reported excretion from schizophrenic subjects to be 2·7 times that from normal subjects, the difference being statistically significant. This contrasts with the findings of the present study, summarized in Figure 4. From this Figure it is clear that xanthine excretion is very variable in both normal and schizophrenic subjects, the extreme values among the 28 people quoted differing by a factor of 5. This readily gives scope for the finding of groups from whom excretion differs 2·7-fold. Indeed a 2-fold difference in excretion was found between two of the subgroups examined in the present study. Excretion by the female schizophrenic patients differed by this factor from that of the male control subjects. However, the male control subjects showed only a quite small difference from the male schizophrenics with whom they were matched in age, weight and in the collection and analysis of the specimens. Also, values from

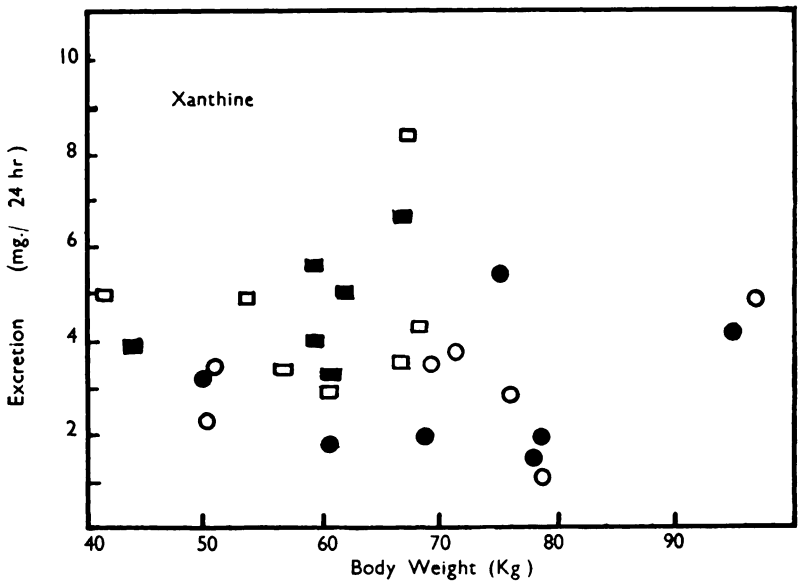

FIG. 4.—Daily excretion of xanthine by the subjects described in Figure 3; symbols and other details as described in Figure 3.

the female schizophrenics were very close to the female control subjects with whom they were similarly matched.

The latter observation gives a practical demonstration of the virtues of the experimental design chosen in the present study. To see whether illness conditions excretion, it is clearly most valuable to match a particular patient with an individually chosen control subject and to collect and analyse the paired specimens together. This chosen design minimized the likelihood of finding artefactual differences between schizophrenic and control subjects. Understandably, it did not control so effectively the other factors examined. In particular, the temporal sequence in the study, which was such that most of the women were examined before the men, made necessary the subsidiary study of Table XI. This probably would not have been necessary had the main investigation alternated the examination of matched pairs of women with matched pairs of men. Comparable details of experimental design are not reported by Kishimoto (1958) and no opinion is therefore expressed as to why his results differ from our own, except that the experimental sequence and physical differences between subjects can greatly influence findings.

The present study of urinary purines in schizophrenia goes much beyond that of Kishimoto (1958) in that the five or six other compounds of Table XI were examined with the same experimental precautions as have been described in relation to xanthine. In none was significant difference found to be associated with the illness. Observations on other compounds, and further studies of the effects of treatment, will be reported subsequently.

SUMMARY

1. Methods for repetitive determination of six endogenous purines in urine have been evaluated by recovery studies. They have been applied to the measurement of hypoxanthine, xanthine, 1- and 7-methylguanines, adenine, 8-hydroxy-7-methylxanthine and guanine in one or more 24-hour specimens from 28 subjects.

2. Ten of these subjects were suffering from severe and clearly defined schizophrenic illnesses. They were matched one by one with 10 normal persons in age, weight and sex, and the collection and analysis of specimens from each patient carried out together with that from the corresponding normal person. Urinary creatinine was also measured.

3. No differences were found between schizophrenic and normal groups in excretion of the compounds named. This applied to xanthine, the excretion of which has been reported to be abnormal in schizophrenia. Those who were highest or lowest in excretion of a particular compound were not of unusual mental status.

4. Physical characteristics were however found to condition purine excretion. This was marked in the case of hypoxanthine, excretion of which increased with increase in body weight. Excretion of hypoxanthine was also greater in men than it was in women of similar weight. This variation with sex was found in most of the compounds studied.

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