cirulating hormone is inactive. The substance which
inactivates the circulating insulin in acromegalic patients
may be similar to, if not identical with, the insulin
inhibitor extracted from normal plasma by Vallance-
Owen et al. (1959a and b); this substance is not present
in the plasma of hypophysectomised patients, and its
inhibitory action is disproportionately reduced by dilution.
Thus the abnormal insulin effects produced by diluted
plasma from male acromegolics could be due to the
presence in undiluted plasma of large amounts of an
insulin inhibitor whose effects are abolished by plasma
dilution; such an inhibitor has yet been isolated or
identified. The abnormal amount of insulin present in undiluted
plasma may be necessary to compensate for the abnormal
resistance of otherwise sensitive peripheral tissues. This
hypothetical, though it may account for the high levels of
activity observed in male subjects, does not explain why
undiluted and diluted plasma from comparable female
subjects which do not have essentially normal levels of activity.
Jefferson (1959) has shown that the rat-diaphragm
method of insulin assay is "sensitive but not very precise"
—an assessment with which Wilbrandts and Green
(1959) agree. Concentrations of the order of 100
micro-units per ml were easily detectable in the present experi-
ments, though in lower concentrations (10 micro-units per ml) insulin did not always produce significant
effects in individual experiments. The insulin equivalent
value deduced from the effects produced by individual
plasma specimens must be regarded as only approximate:
the correct value may be anything from a half to twice
the estimated figure. Thus, though this method of assay is
very sensitive, its inaccuracy may be responsible for
its failure to provide information of clinical significance
in individual cases. There is considerable evidence (Green
et al. 1952, Randle 1954c, Vallance-Owen and Hurlock
1954, Randle and Taylor 1958) that the biological
effects produced by plasma are due to the insulin which it
contains, but other substances may also be present which
grow or diminish its effects. Further investigations,
similar to those of Field and Stetten (1950) and Vallance-
Owen et al. (1959a and b) and aimed at the detection and
identification of such inhibitors and augmentors in plasma,
might yield more useful information concerning the
abnormal carbohydrate metabolism in human disease than
the less specific plasma-insulin assays carried out in the
past.

Summary
Insulin assays were carried out on plasma drawn from
18 normal subjects, 14 acromegolics, 7 patients with
islet-cell tumours of the pancreas, and 3 children with
idiopathic spontaneous hypoglycaemia.
Normal undiluted plasma has a biological effect equi-
valent to that of insulin in a concentration of approxi-
mately 70 micro-units per ml. The insulin equivalent
of plasma diluted at least 4-fold is about 200 micro-units per ml.

Plasma from female acromegolics, assayed in the
undiluted and diluted form, has essentially normal
biological activity, while at all levels of dilution plasma
from male acromegolics is significantly more active. It is
suggested that the latter contains high concentrations of
an insulin inhibitor.
Plasma from 3 of the cases of islet-cell tumours was
abnormally active. It is concluded that normal levels of
activity in suspected cases do not contraindicate this
diagnosis.

Normal levels of activity were found in the 3 cases of
spontaneous hypoglycaemia of infancy.

Whereas knowledge of plasma-insulin activity may help
to explain the underlying causes of abnormal carbohydrate
metabolism in some pathological conditions, assays in
individual cases seldom provide information useful to
the clinician.

I should like to thank those clinicians of other hospitals
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LEUCINE AND PELLagra

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Pellagra has long been known to be predominantly a disease of maize-eaters. The association of pellagra with maize consumption has been attributed, among other factors, to the low tryptophan content of maize, and to the poor availability of its nicotinic acid. The disease is rare in areas where rice constitutes the staple. In almost ten years' experience with many cases of nutritional deficiencies, we have seen only 4 classical cases of pellagra among the rice-eating population of Coonoor.

On the other hand, in Hyderabad, a city nearly five hundred miles further north, we frequently encounter cases of pellagra. Careful examinations of the distatites of the poor segments of the population in Coonoor and Hyderabad failed to reveal any striking differences with regard to the intake of different nutrients; but, whereas rice is the sole staple in Coonoor, the diets of the poor segments of the population in Hyderabad invariably include the millet Sorgum vulgare (jowar). In practically every case of pellagra investigated by us in Hyderabad, a history of regular consumption of jowar with or without

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The amino acid composition of maize, jowar, and rice was obtained. In only one case was there a history of consumption of maize in addition to jowar and rice.

Comparison of the chemical composition of rice, jowar, and maize (see accompanying table) shows that the niacinic-acid content of jowar is similar to that of rice. The reported tryptophan content of different strains of jowar varies widely, certain strains having nearly as high a content of the aminosacids as is found in rice while certain others have low values as in maize (Carpenter and Kodick 1950). Both jowar and maize have, however, one common feature with regard to their aminosacids composition—namely, a high content of leucine. Elvehjem (1950) reported that the dietary supplementation of leucine at 1% level caused retardation of growth in rats subsisting on low-protein diets (9% casein), but that such growth retardation was not observed if the dietary protein intake was raised to 15%. The average daily protein intake in the diets of the pellagra patients investigated here was of the order of 45 g. (5% protein), the protein being mainly derived from cereals including jowar. The possible role of aminosacids imbalance resulting from relative excess of leucine in the pathogenesis of pellagra was investigated, and the preliminary results are described here.

Experimental

Effect of Administration of Leucine on Urinary Excretion of N-methyl Nicotinamide (N.N.M.) in Healthy Volunteers and in Pellagrians

2 normal healthy male subjects were put on a basal diet providing roughly 10% protein, and their urinary N.N.M. excretion was determined daily by the method of Carpenter and Kodick (1950). The basal diet was continued until a fairly stable level of N.N.M. excretion was attained. The volunteers were then given a daily supplement of 5 g. L-leucine along with one meal in a single dose, for seven days, and their urinary N.N.M. excretion was determined daily. N.N.M. levels were determined by the method of Sweeney and Hall (1951) (with some modifications) before, during, and after leucine supplementation. Similar studies were also carried out in 3 patients with pellagra.

To determine whether the changes in urinary N.N.M. excretion brought about by leucine administration could be influenced by isoleucine, 2 other healthy male subjects were investigated. These subjects also received 5 g. leucine daily for three days after the initial stabilisation period, but the four days thereafter they received 2 g. of ni-isoleucine daily in addition to the 5 g. L-leucine.

To facilitate the quantitative interpretation of the changes in N.N.M. excretion in terms of nicotinic-acid metabolism, two healthy volunteers, who had been earlier investigated with leucine as above, received a 15 mg. oral dose of nicotinic acid and the increase in their urinary excretion of N.N.M. was determined.

Fig. 1—Effect of leucine supplementation on urinary N.N.M. excretion in a healthy subject.

Fig. 2—Effect of isoleucine and isonitrogenous substitution of rice by jowar on urinary N-N-methyl Nicotinamide Excretion

3 pellagrians were put on a basal diet providing 10% protein derived from rice and wheat, and the daily urinary N.N.M. excretion was determined. After the N.N.M. excretion had stabilised, rice in the diet was replaced by jowar and the daily urinary N.N.M. excretion on this diet was studied. Actual analysis of the two diets revealed that the rice-wheat diet in the 3 cases provided 7.3, 7.9, and 7.6 mg. nicotinic acid daily, while the jowar-wheat diet provided 7.8, 8.1, and 7.5 mg. nicotinic acid daily.

Effect of Administration of Large Amounts of Leucine in Pellagrians

2 patients with pellagra were placed on a basal diet supplying 45 g. of protein daily, the protein being mainly derived from jowar. After a preliminary stabilisation period these patients were given 10 g. L-leucine—twice daily in 1 case and thrice daily in the other—along with the principal meals. The urinary N.N.M. excretion was studied daily, and their clinical condition was carefully watched.

In this study the cases of pellagra were chronic, and the patients were not seriously ill. The patients volunteered for the investigation. We were confident that any deleterious effect of administering leucine could be corrected effectively and promptly by administration of nicotinic acid.
Results

Effect of Administration of Leucine on Urinary Excretion of N.M.N.

The results of this study are illustrated in figs. 1–3. In all subjects investigated, both healthy volunteers and cases of pellagra, urinary excretion of N.M.N. increased immediately after administration of leucine. The rise in N.M.N. excretion brought about by 5 g. L-leucine daily represented an increase of nearly 50% over the basal level. Within a few days after the withdrawal of leucine, N.M.N. excretion returned to the basal level in all cases. There were no significant changes in the blood-nicotinic-acid levels. Determination of blood-nicotinic-acid levels in the same individual on a standard diet showed wide fluctuations, the values ranging from 600 µg. per 100 ml. to 1000 µg. per ml.

The increase in N.M.N. excretion brought about by leucine did not appear to be influenced by simultaneous administration of isoleucine (fig. 3). The administration of isoleucine did not alter the N.M.N. excretion pattern.

In the 2 healthy volunteers in whom the changes in N.M.N. excretion after administration of nicotinic acid were also studied, it was found that the administration of 15 mg. nicotinic acid resulted in an increase of urinary N.M.N. excretion of the order of 8.3 mg. or 17.5 mg. This suggests that the average increase of 1.7 mg. in N.M.N. excretion brought about by the administration of 5 g. of L-leucine reflects approximately an additional 3.5 mg. of nicotinic acid metabolised daily.

Effect of Isoleucine and Isornitrogenous Substitution of Rice by Jowar on Urinary N.M.N. Excretions

The effect of substituting jowar for rice in a typical case is illustrated in fig. 4. The replacement of rice by jowar resulted in a prompt increase in urinary N.M.N. excretion over the basal level observed in the “rice period”. This increase was maintained as long as jowar was administered. After the withdrawal of jowar and the readministration of rice, N.M.N. excretion returned to the original low level.

It was calculated that the daily amount of leucine contained in the jowar diet was of the order of 5.3 g. The increase in N.M.N. excretion observed was also of the same order as that obtained with 5 g. L-leucine daily. Supplementation with isoleucine did not influence the N.M.N. excretion pattern on the jowar diet.

Effect of Administration of Large Amounts of Leucine in Pellagrin

In the course of the studies, 5 g. L-leucine daily was administered to 4 patients with pellagra for seven days. This was not associated with any appreciable effects on the clinical picture. The clinical features in 2 other cases in which larger amounts of leucine were given daily were as follows:

Case 1.—The patient was admitted with classical extrinsic skin changes, oral manifestation of vitamin-B-complex deficiency, and diarrhoea. On admission, apart from a feeling of anxiety, he did not seem to be mentally abnormal. He answered questions promptly and correctly. He continued in this state for four days, during which he was maintained on the basal diet to attain a stable level of N.M.N. excretion. On the fifth day he was given 30 g. of L-leucine in divided doses of 10 g. each at six-hourly intervals. Towards the end of the day the patient was found to behave in an agitated manner, became incoherent in his talk, and to exhibit delusions of grandeur. The periods of excitement were found to alternate with short periods of severe depression. An additional dose of 10 g. of L-leucine was administered next morning, and by noon the patient became uncontrollable and violent. At this stage leucine was discontinued and he was given nicotinic acid intramuscularly. Within forty-eight hours after the initiation of nicotinic-acid therapy the patient’s mental state had reverted to normal.

Case 2.—On admission the patient exhibited the classic skin changes and severe glossitis and had diarrhoea. He appeared alert and answered questions intelligently; he did not show muscular changes. He was placed on the basal diet and after the period of stabilisation he was given 29 g. L-leucine daily in divided doses of 10 g. each along with the periods meals. Leucine administration was continued for sixteen days and was then stopped.

Unlike all other cases of pellagra seen by us, in whom clinical improvement resulted from admission to hospital, even on the basal diet without nicotinic-acid supplementation the patient did not improve clinically; but there was no evidence of worsening of the skin condition or glossitis. Three days after leucine was started, the diarrhoea became more severe and the patient complained of insomnia. On the sixth day the patient became severely depressed and apprehensive; his answers to questions were no longer prompt. When leucine was stopped on the sixteenth day the patient seemed slightly disoriented. Three days after cessation of leucine administration, his mental condition became suddenly worse. He became violent with paranoid delusions and had to be forcibly restrained; his speech was incoherent. At this stage treatment with nicotinic acid was initiated and within forty-eight hours the mental condition returned to normal.

The urinary excretion of N.M.N. was followed throughout the period. Immediately after leucine administration started there was an increase in N.M.N. excretion, which was maintained throughout the period of leucine supplementation. Three days after leucine was discontinued, when the patient’s mental condition had worsened, N.M.N. excretion was still at the same high level as during the period of leucine administration. It was, however, noticed that the increase in the level of N.M.N. excretion brought about by the feeding of 20 g. of L-leucine in this case was not significantly greater than that observed with 5 g. L-leucine in the healthy subject and other cases of pellagra.

During the period of leucine administration, the patient lost nearly 2.5 kg. body-weight. Within three days after leucine was stopped the weight loss was arrested, and thereafter the patient started gaining weight.

Discussion

While pellagra has long been known to be associated with maize consumption, in the present study the disease has been found to be related to the consumption of the millet “jowar” (Sorghum vulgare). The increase in urinary N.M.N. excretion brought about by the replacement of rice by jowar suggests that the greater prevalence of pellagra in jowar-eaters than in rice-eaters may not be...
The increase in N.M.E. excretion brought about by leucine requires clarification. This increase is unlikely to be due to greater availability of nicotinic acid, because both maize and jowar, which are rich in leucine, have been associated with increased prevalence of pellagra. Apparently the aminoisobutyric imbalance caused by relative excess of leucine in diets which are already marginal with regard to nicotinic acid, this may be an important factor.

It would be premature to draw conclusions from these studies as to the role of leucine in the pathogenesis of pellagra. Case 2, in whom the effect of large doses of leucine was investigated, lost nearly 2.5 kg. of body-weight in a fortnight during which leucine was administered. This possibly suggests that the increase in N.M.E. excretion brought about by leucine may not be a specific effect of leucine on nicotinic acid metabolism, but reflects increased tissue catabolism due to the aminoisobutyric imbalance. On the other hand, 6 subjects—2 healthy and 4 pellagrins—in whom the effect of leucine was investigated, only 2 with pellagra (including case 2) showed reduction in body-weight; in the remainder the increase in urinary N.M.E. excretion was associated with a stationary body-weight.

SUMMARY

Oral administration of 5 g. of leucine daily brought about an increase in urinary excretion of N-methyl nicotinamide excretion in healthy subjects and in patients with pellagra. This increase was not influenced by isoleucine. Isocaloric replacement of rice by jowar brought about an increase in the urinary N-methyl nicotinamide excretion in healthy subjects and in patients with pellagra. This increase also was unaffected by isoleucine.

Oral administration of 20–30 g. of leucine daily to 2 patients with pellagra was associated with temporary deterioration of their mental condition, which was reversed when leucine was discontinued and nicotinic acid was administered.

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