

amounts ranging between 0.4–2.6 mg cyclohexylamine/8 h urine sample. This substance was undetected in the urine of the remaining subjects.

(b) In fifty-two other normal volunteers (forty men, twelve women), 24 h urine samples were obtained before, and after, each received 50 mg/kg body-weight of sodium cyclamate administered as before in pure lemon juice. Cyclohexylamine was detected in the urine of eight of the subjects in amounts from 0.5 to 4.6 mg/24 h urine, but was absent from the urine of the other forty-four subjects.

(c) Ten adult volunteers, three of whom were 'cyclohexylamine excreters' in experiment (b) above, received 1 g sodium cyclamate/d for 28 d. Daily 24 h urine collections were obtained from each subject, and analysed for cyclohexylamine. The amounts of this substance in the urine of the 'excreters' increased progressively with time to about the 9th day, when peak values of approximately 7–8 mg/24 h urine were found. The cyclohexylamine values then plateaued out, with some fluctuation, between 4–6 mg/24 h urine. In three other subjects cyclohexylamine was detected in the urine on some days in amounts between 0.6–1.7 mg/24 h urine. In the other four subjects, cyclohexylamine was not detected in the urine throughout the 28 d period of study.

The author would like to thank Professor A. Kekwick for encouragement and the subjects for co-operation. Dr E. L. Harris of Abbott Laboratories Ltd, kindly provided supplies of sodium cyclamate.

REFERENCE

Kojima, S. & Ichibagase, H. (1966). *Chem. Pharm. Bull., Tokyo* **14**, 971.

The Two Hundred and Sixteenth meeting of the Nutrition Society was held in the London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1, on Thursday, 4 December 1969, at 16.30 hours, when the following papers were read:

Quantitative aspects of biohydrogenation in the rumen of the lactating goat. By R. BICKERSTAFFE, D. E. NOAKES and E. F. ANNISON, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

Unsaturated fatty acids liberated during the rapid hydrolysis of complex lipids in the rumen suffer extensive hydrogenation and isomerization (see Garton, 1966). We have used lactating goats prepared with re-entrant cannula placed just inside the duodenum to allow the quantitative collection and analysis of material leaving the abomasum (Harrison & Hill, 1962). The goats, maintained on a high-fat ration containing 5% soya-bean oil, were fed twice daily or continuously, at intervals of 1 h throughout 24 h.

The feeding regime markedly influenced duodenal flow rates. On continuous feeding, the volume of digesta was approximately 62% of that obtained from the same animal fed twice.

The intakes of dietary octadecanoic, octadecenoic, octadecadienoic and octadecatrienoic acids on both regimes were 4.2, 23.5, 49.0 and 16.0 g/24 h. The corresponding values for the amounts of these acids entering the duodenum were 81.0, 24.7, 4.9 and 0.7 g/24 h in the twice daily fed goats, and 81.7, 20.0, 9.0 and 1.8 g/24 h in the continuously fed goats. In both instances, the total lipid output from the rumen exceeded that supplied in the diet, reflecting synthesis of fatty acids by the rumen microflora.

In the twice daily fed goats, 16% of the duodenal octadecenoic acid was in the *cis* form. The corresponding value for the continuously fed goat was 33%, and further analysis showed that 43% and 53% respectively of these fractions were oleic acid (*cis*-octadeca-9-enoic acid), the remainder comprising isomers in which the double bond occurred in almost every possible position. The *trans*-octadecenoic acid fractions from animals maintained on both feeding regimes comprised 1-2% elaidic acid (*trans*-octadeca-9-enoic acid), 54-57% octadeca-11-enoic acid and the remainder a mixture of all possible isomers.

Analysis of the duodenal octadecadienoic acid showed that linoleic acid (*cis*, *cis*-octadeca-9,12-dienoic acid) accounted for 95% of this fraction on both feeding regimes, although only 10-15% of the linoleic acid escaped hydrogenation.

REFERENCES

- Garton, G. A. (1966). *Wild Rev. Nutr. Diet.* **7**, 225.
Harrison, F. A. & Hill, K. J. (1962). *J. Physiol., Lond.* **162**, 225.

Some effects of feeding supplements of maize meal and sodium bicarbonate upon the digestion of forage cellulose by sheep. By D. F. OSBOURN, R. A. TERRY, S. B. CAMMELL and G. E. OUTEN, *Grassland Research Institute, Hurley, Berkshire*

Terry, Tilley & Outen (1969) have demonstrated a progressive decline in the rate and quantity of forage cellulose digested *in vitro* as the pH of the system was reduced below pH 6.8. In this present experiment we have examined the changes occurring in the rate and extent of digestion of forage cellulose by sheep when the pH of the digesta in the reticulo-rumen was altered by the feeding of supplements of maize and sodium bicarbonate.

Groups of four Suffolk × Half-bred wether sheep, mean live-weight 46 kg, were fitted with rigid cannulas and fed either twice or 24 times each day. Both groups were fed a restricted quantity of chopped dried grass at the rate of 38 g dry matter/kg $W^{0.73}$ per 24 h (diet F) during the first period of 32 d duration. In a second similar period the sheep were fed the same quantity of dried grass plus 20 g DM/kg $W^{0.73}$ per 24 h, of pelleted maize meal (diet FM). In a third period the sheep were

fed the same quantity of dried grass daily plus 26.4 g DM/kg $W^{0.73}$ per d of a pelleted mixture of 100 parts of maize meal to 32 parts of sodium bicarbonate (diet FMB). This diet contained the same quantity of hay and maize as diet FM and the NaHCO_3 comprised 10.0% of the total intake.

The effect of frequency of feeding was not large, and is not discussed here. Supplementation of the dried grass with maize meal depressed both the mean rumen pH and the rate of disappearance of cotton threads suspended in the rumen (Table 1). The addition of sodium bicarbonate (diet FMB) restored the rumen pH and the rate of cotton thread digestion to the levels observed with diet F; the consumption of water was also increased compared with the other two diets.

Table 1. Mean values of some observations made upon sheep fed diets of dried grass (F), dried grass plus maize meal (FM) and dried grass plus maize meal and sodium bicarbonate (FMB)

| | F | Diet FM | FMB | Standard error of mean | FM v. FMB <i>P</i> |
|--|------|------------|------|------------------------------|-------------------------|
| Rumen pH (mean over 24 h) | 6.57 | 6.04 | 6.51 | ± 0.085 | 0.01 > <i>P</i> > 0.001 |
| Time to digest 25% cotton thread (h) | 26.8 | 42.0 | 26.8 | ± 2.94 | 0.01 > <i>P</i> > 0.001 |
| Retention of stained forage, (80-5% excretion times (h) | — | 94.0 | 73.7 | ± 6.29 | 0.10 > <i>P</i> > 0.05 |
| Digestibility of cellulose (%) | 81.1 | 77.7 | 77.3 | ± 0.61 | Not significant |
| Water consumption (l/d) | 1.44 | 1.64 | 4.26 | ± 0.182 | 0.001 > <i>P</i> |

Correlation coefficients ($n=24$) between the time taken to digest 25% of the cotton threads and (a) the arithmetical mean of the hourly pH observations over a 24 h period or (b) the sum of the squares of the deviations of the hourly pH from 6.8 were -0.747 and $+0.773$ respectively.

Despite the differences observed in cellulolytic activity within the rumen, the extent of cellulose digestion was similar on the three diets. This contrasts with the *in vitro* results, and may in part be attributable to different retention times, as measured with diets FM and FMB. The possible effect of these treatments on level of voluntary intake is being investigated.

REFERENCE

- Terry, R. A., Tilley, J. M. A. & Outen, G. E. (1969). *J. Sci. Fd Agric.* **20**, 317.

The production of amines in the alimentary tract of the pig in relation to dietary lactobacilli, weaning and post-weaning diarrhoea. 1. *In vivo* studies of intestinal and urinary amines. By P. PORTER, R. KENWORTHY and I. R. HILL, *Unilever Research Laboratory Colworth/Welwyn, Colworth House, Sharnbrook, Bedford*

Changes in the intestinal flora of the pig are known to take place following dietary change or weaning, frequently with the proliferation of pathogenic *Escherichia coli* serotypes. Considerable emphasis has been placed on the study of toxic or immune reactions of intestinal flora upon the pig but little attempt has been made to study the changes in metabolic activities of the flora associated with weaning and post-weaning diarrhoea.

Michel (1962) has indicated that the microbial flora of the pig intestine was active in decarboxylating amino acids, and there is circumstantial evidence that the catabolic activity of the intestinal flora affects the performance of the host. The growth-promoting effects of antibiotics may be related to the suppression of catabolic activity with a consequent reduction in the level of intestinal amines (Larson & Hill, 1960).

In the present studies pigs were weaned abruptly at 3 weeks on to cow's milk reconstituted from spray-dried powder fed as such, or after 48 h incubation with *Lactobacillus acidophilus*. Intestinal amines were investigated in animals slaughtered in the immediate pre- and post-weaning period and urinary heterocyclic amines were used to monitor the formation of diamines due to microbial action in the intestine of the intact animal.

The level of excretion of urinary heterocyclic amines increased immediately after weaning and again during the period of diarrhoeic faeces. The overall production of diamine within the alimentary tract, as reflected by heterocyclic amine excretion, was no indication of the predisposition to diarrhoea since normal and scouring animals may excrete similar levels.

The site of production of amine within the alimentary tract may be an important factor. In severely diarrhoeic animals the small intestine was the main site for production of amines whereas in clinically unaffected animals there was only a low level of amine production in the small intestine. The effect of including lactobacilli in the diet was investigated and this apparently produced a transfer of catabolic activity from the small intestine to the colon during the diarrhoeic phase.

Ion-exchange studies of caecal extracts together with the examination of the eluates by thin-layer chromatography demonstrated that the predominant diamines produced in the alimentary tract were cadaverine and putrescine.

REFERENCES

- Larson, M. L. & Hill, E. G. (1960). *J. Bact.* **80**, 188.
Michel, M. C. (1962). *Amino Acides* **5**, 157.

The production of amines in the alimentary tract of the pig in relation to dietary lactobacilli, weaning and post-weaning diarrhoea. 2. In vitro studies of the catabolic activity of the small intestinal microflora.

By I. R. HILL, R. KENWORTHY and P. PORTER, *Unilever Research Laboratory Colworth/Welwyn, Colworth House, Sharnbrook, Bedford*

The inclusion into diets of milk products soured by lactobacilli or lactic acid

has long been considered beneficial in microbially associated enteritis of the human. Their use as treatments for, or prophylactics against, enteric disease states stems from the many recorded instances of association between intestinal disease and proliferation of members of the Enterobacteriaceae, the rationale being that these diseases occur due to changes in the normal balance of the intestinal flora and that lactobacilli or their products act as stabilizing agents. However, experiments which have reported successful therapy by reducing the numbers of a particular microbial genus or species have not been convincing.

Coincident to the study of Porter, Kenworthy & Hill (1970) we have examined the effect of dietary lactobacilli on the *in vitro* deaminative and decarboxylative activities of the small intestinal microflora, in relation to weaning and post-weaning diarrhoea. Pigs were weaned abruptly at 21 d of age on to a diet of milk and milk incubated with lactobacilli, and measurements of catabolic activity were made on small intestinal digesta with and without the supplementation of L-arginine, L-histidine, L-lysine and L-ornithine.

An increase in decarboxylative and deaminative activities was observed within 48 h of weaning in animals fed both diets. Decarboxylative activity was highest, and deaminative activity lowest, in the digesta from pigs fed milk without lactobacilli. During a later period, when diarrhoea was observed, there was a second peak of decarboxylative activity in the digesta of animals fed both diets; but deaminative activity only increased in animals receiving dietary lactobacilli. In animals fed dietary lactobacilli the severity and duration of diarrhoea was reduced. Thin-layer chromatography has been used to indicate the variation in *in vitro* arginine metabolism by pure cultures of *E. coli* isolated from pigs. Decarboxylases were found to be inactive at pH 6 and above; whilst in *in vitro* studies of intestinal digesta decarboxylative activity was evident at up to pH 7.5.

Continuous administration of dietary lactobacilli failed to induce any consistent changes in the small intestinal microflora.

REFERENCE

- Porter, P., Kenworthy, R. & Hill, I. R. (1970). *Proc. Nutr. Soc.* **29**, 13A.

Effects of fractions from navy beans (*Phaseolus vulgaris*) on germ-free and conventional chicks. By D. HEWITT and MARIE E. COATES, *National Institute for Research in Dairying, Shinfield, Reading* and M. L. KAKADE and I. E. LIENER, *Department of Biochemistry, College of Biological Sciences, University of Minnesota, St Paul, Minnesota, USA*

Hewitt & Coates (1969) showed that the growth-depressing activity of raw navy-bean meal (NBM) was significantly less in germ-free (GF) than in conventional (CV) chicks. Pancreatic enlargement was similar in both environments. Comparisons with soya-bean meal suggested that the pancreatic enlargement was related to the trypsin inhibitor but growth depression was due to different factor(s).

Kakade & Evans (1965) prepared various fractions from raw NBM; F₃ depressed growth and had high trypsin-inhibitor activity; F₄ depressed growth much more severely but had low trypsin-inhibitor activity. These fractions have now been tested on GF and CV chicks given a diet containing 50% NBM supplemented with 10% casein, arginine, methionine and glycine. The mean body-weight (g) and pancreas weight (g/100 g body-weight) of groups of ten chicks at 2 weeks of age were:

| | Body-wt | | Pancreas wt | |
|--------------------------------|---------|-----|-------------|------|
| | GF | CV | GF | CV |
| Raw NBM | 116 | 65 | 0.68 | 0.71 |
| Heated NBM | 132 | 125 | 0.36 | 0.46 |
| Heated NBM+0.8% F ₃ | 133 | 108 | 0.61 | 0.77 |
| Heated NBM+1.5% F ₄ | 125 | 92 | 0.36 | 0.50 |

In both environments F₃ caused a significant ($P < 0.01$) enlargement of the pancreas (indicating trypsin inhibition) comparable with that produced by raw NBM but, as F₃ depressed growth significantly ($P < 0.02$) only in CV chicks, it appears that trypsin inhibition *per se* is not directly responsible for poor growth. The effect of environment was also observed with F₄, which depressed growth more severely than F₃, but only in CV chicks. It is clear that the growth-depressing action of F₃ and F₄ is mediated through the gut microflora, possibly by microbial production of toxins from non-toxic precursors or by encouraging the establishment in CV chicks of organisms detrimental to the host's well-being. The slightly poorer growth of GF chicks on raw NBM may indicate a further toxic factor, not represented in F₃ or F₄, the action of which is independent of the presence of micro-organisms.

REFERENCES

- Hewitt, D. & Coates, M. E. (1969). *Proc. Nutr. Soc.* **28**, 47A.
 Kakade, M. L. & Evans, R. J. (1965). *J. agric. Fd Chem.* **13**, 450.

The effect of intestinal coccidiosis upon energy utilization in the chick.

By A. H. SYKES, *Wye College (University of London), Ashford, Kent*

Infection with *Eimeria acervulina* in the growing chick results in a reduction of body-weight gain or an actual loss of weight depending upon the size of the infection. These weight changes are accompanied by a fall in feed intake but uninfected, pair-fed birds do not lose as much weight and show a more rapid return to the normal growth rate. A closer examination of the differences between infection and feed restriction was made in a series of balance trials on groups of 4-week-old male chicks over a period of 10 d. One group was infected with 5 million oocysts of

E. acervulina and daily measurements were made of feed intake, and hence energy and nitrogen intakes by calculation, body-weight and energy and nitrogen excretion.

The energy and nitrogen intakes of the infected and pair-fed birds fell with the declining feed intakes reaching a minimum on days 4 and 5 and then increased quite sharply on day 7. The daily percentage retention also fell in both these groups compared with the control, from which it might appear that the effects of coccidiosis could be accounted for solely on the basis of reduced feed intake. However, when the total energy balance over 10 d was determined it was found that the infected birds had retained 871 kcal out of 1175 consumed and the pair-fed birds had retained 676 kcal out of 1050 consumed but the body-weight gains over the period were 68 g and 186 g respectively. Uninfected control birds on *ad lib.* feeding retained 1310 kcal out of 1755 consumed and gained 185 g.

Thus although reduced feed intake contributes to the weight loss during an infection, it is also clear that the subsequent utilization of the absorbed energy is impaired, perhaps as a result of a circulating toxin.

Diet, leisure activity and skinfold measurements of sedentary men. By

JEAN W. MARR, JULIA GREGORY, T. W. MEADE, M. R. ALDERSON* and J. N. MORRIS, *MRC Social Medicine Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1*

In an attempt to validate a short self-completion retrospective record of physical activity other than at work, i.e. during leisure time (Yasin, Alderson, Marr, Pattison & Morris, 1967), the weighed inventory method of dietary assessment (Garry, Passmore, Warnock & Durnin, 1955) and measurement of skinfold thickness were used as independent measures. Each 5 min of recorded activity were assessed for levels of energy expenditure and given a 'score' from 1 to 5. The total score for an individual was the sum of these separate scores and, the range of scores being divided into thirds, the men were graded as active, middle or inactive. It was postulated that if the record were measuring levels of activity a higher calorie intake, and also smaller skinfold measurements, would be found in the 'active' group of men than in the 'inactive'.

Ninety-five male civil servants aged 40–55 years engaged in office work in central London completed a 2 d retrospective record in which they listed chronologically their leisure-time activities. The following week each man was interviewed by a dietitian, when instructions were given for keeping a weighed record of food eaten at home for 3 d. Sample helpings of canteen foods were weighed by the investigators, and the contents of meals taken out estimated. On completion of the dietary survey each man was given a medical examination (T.W.M.) including skinfold measurements using the Harpenden calliper, and blood was taken for lipid estimation. The record of leisure activity and 3 d dietary survey were repeated 3 months

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Table 1. Mean daily energy consumption, skinfold thickness and serum lipids in ninety-five male civil servants

| Variable | Level of activity of men | | | r* |
|---------------------------------------|--------------------------|--------|----------|---------|
| | Active | Middle | Inactive | |
| Energy: kcal | 2925 | 2837 | 2608 | 0.31** |
| kcal/kg | 40.2 | 37.6 | 33.2 | 0.41*** |
| Triceps } Skinfold (mm) | 9.3 | 10.8 | 10.7 | -0.08 |
| Subscapular } | 12.8 | 13.8 | 16.3 | -0.24** |
| Suprailiac } | 8.8 | 10.2 | 12.1 | -0.25** |
| Wt (kg) | 73.5 | 76.4 | 79.2 | -0.25** |
| Serum cholesterol (mg %) [†] | 242 | 250 | 260 | -0.16 |
| Triglycerides (mg %) [†] | 113 | 142 | 140 | -0.11 |
| Average age (years) | 47 | 47 | 45 | |
| No. of men | 30 | 33 | 32 | |

*Correlation with activity score: ** $P < 0.01$; *** $P < 0.001$.

[†]Determined at St George's Hospital, London.

later. Eighty percent of the men co-operated in all the phases of the study. Men on prescribed diets have been excluded.

Results are given in the table; briefly, they indicate some measure of validity of the record of leisure activity.

We are greatly indebted to the men who co-operated in the study and also to our colleagues in the MRC Social Medicine Unit for advice and criticism.

REFERENCES

- Garry, R. C., Passmore, R., Warnock, G. M. & Durnin, J. V. G. A. (1955). *Spec. Rep. Ser. med. Res. Coun.* no. 289.
- Yasin, S., Alderson, M. R., Marr, J. W., Pattison, D. C. & Morris, J. N. (1967). *Br. J. prev. soc. Med.* **21**, 163.

The effect of 10 weeks consumption of a 75% sucrose diet on the incorporation of [U-¹⁴C]fructose into the serum glycerides of male and female baboons. By M. H. JOURDAN, Department of Physiology, Guy's Hospital Medical School, London, SE1

Coltart (1968) has shown that when baboons are kept on a diet containing 75% sucrose for 13 weeks, the specific activity in the serum glycerides following a meal of [U-¹⁴C]sucrose is greater than before the diet in both male and female baboons, although the increase was found to be greater in male baboons. The present study was designed to determine if a similar increase in the glyceride specific activity was found when [U-¹⁴C]fructose was given intravenously at the beginning and end of a 10-week period of 75% sucrose diet.

Six male and six female baboons, all sexually mature, were used in the experiment. After an overnight fast, each animal was lightly tranquilized and given 0.5 g uniformly labelled [¹⁴C]fructose/kg body-weight intravenously, as a 20% solution, over 3 min. Venous blood was collected before the injection and at timed periods up to 5 h following the injection. The baboons were then kept on a diet containing 75% sucrose and 18% casein, with salt and vitamin supplements, for 10

weeks. Fasting venous blood samples were obtained weekly, and at the end of the period the [U- ^{14}C]fructose tolerance test was repeated.

The serum glyceride concentrations were determined gravimetrically after separation on thin-layer silica and the ^{14}C activity was measured in a liquid scintillation counter. Serum fructose concentrations were determined using the method of Roe (1934).

It was found that the fasting serum glyceride concentration rose in both male and female baboons while on the diet, reaching a peak increase at 3 weeks and returning to control values by the end of the dietary period. There was no significant difference between the sexes in this respect.

Although the ^{14}C specific activity in the glycerides of the male baboons following fructose was significantly greater than in the females, no significant change occurred between the beginning and the end of the diet.

It is concluded that although a diet containing 75% sucrose probably increases the rate of intestinal absorption of fructose, it does not alter the ratio of various precursor substances used in the synthesis of serum glycerides. Thus there is no preferential induction of enzyme systems concerned in the conversion of fructose to glycerides.

REFERENCES

- Coltart, T. (1968). PhD Thesis, University of London.
Roe, J. H. (1934). *J. biol. Chem.* **107**, 15.

The body fat in a random sample of adolescent schoolchildren in Glasgow.

By J. V. G. A. DURNIN and M. M. RAHAMAN, *Institute of Physiology, University of Glasgow*

Vitamins B and C and tongue signs in adolescents. By J. P. SAUNDERS, *Royal Army Medical Corps, Shorncliffe* and D. H. NORMAN, *Public Health Department, Hounslow, London* and W. T. C. BERRY, *Department of Health and Social Security, London, SE1*

Yudkin, Norman, Wilkinson & Berry (1970) described an absence of change in fissuring and fungiform papillae in secondary schoolchildren after administration on 103 out of 166 d of vitamins B and C in amounts that were non-pharmacological but adequate to redress any nutritional defect. In the present study, on members of the Junior Infantrymen's Battalion, larger doses were given daily for 15 months with gaps for leave periods. The dose for the first 7 months was thiamine 2 mg, riboflavine 5 mg, nicotinic acid 15 mg, pyridoxine 2.5 mg and ascorbic acid 100 mg, and for the last 7 months 5, 10, 35, 5 and 100 mg respectively. The potent and dummy tablets were fed, nightly, by the sergeant in charge of each platoon, each platoon receiving either one potent or one dummy tablet throughout. The observers were unaware which group received which tablet. Photographic comparisons were made at the beginning and end of the study and at intervals during it.

Eighteen boys completed the period on the potent, and twenty-five on the dummy, tablets. Seven and twelve respectively, had fungiform papillae judged as large and red. In the fed group four remained unchanged, two seemed slightly better, one definitely worse. In the controls one seemed slightly better, one slightly worse, and one definitely worse. Among four 'fed' and six controls fissuring remained unchanged except one control seemed slightly worse. In one control and one 'fed' subject the filiform papillae seemed longer at the end of the study, and in one subject in each group denuded areas were apparent at the close.

In short, increase in dosage and duration did not lead to an outcome any less negative than that already reported by Yudkin *et al.* (1970).

REFERENCE

Yudkin, J., Norman, D. H., Wilkinson, M. E. & Berry, W. T. C. (1970). *Proc. Nutr. Soc.* **29**, 8A.

Absence of effect of thiamine and other vitamins on duration of myotatic response. By T. P. EDDY, *London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1* and J. P. SAUNDERS, *Royal Army Medical Corps, Shorncliffe* and W. T. C. BERRY, *Department of Health and Social Security, London, SE1*

Among young infantrymen fed a vitamin tablet for 15 months (see Saunders,

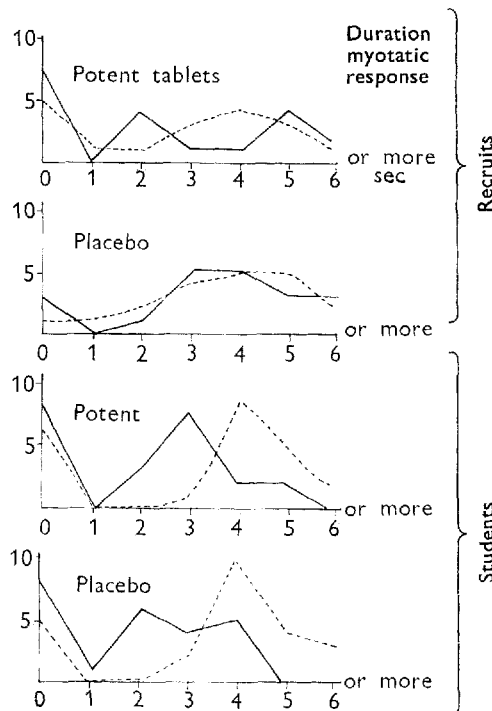


Fig. 1. Response to treatment with vitamins: — — —, at commencement; —, at close.

Norman & Berry, 1970) and among technical college male students fed thiamine 10 mg daily for 5 d per week for two terms, no greater reduction in myotatic response was observed than among controls (Fig. 1).

Whilst the normal distribution of myotatic responses (where these are elicited) has never been established, we suspect that a mode of 4 s, with a fairly symmetrical spread of 3 or 4 s above and below the mode, is physiological and that we were dealing with subjects wholly normal in this respect. We think that the excess of negative values and the shift to the left of values apparent in both series but more in the second pair could result in part from an involuntary tensing of muscle in apprehension of the unavoidable though slight discomfort of the test.

REFERENCE

- Saunders, J. P., Norman, D. H. & Berry, W. T. C. (1970). *Proc. Nutr. Soc.* **29**, 19A.