TABLE IIIa.—*Response of Patients in Group 4—Schizo-affective Psychosis*

Case Type	Much Improved	Improved	No Improvement or Relapsed	Untraced	Total
Depressed ..	2	0	11	1	14
Manic ..	5*	0	2	—	7

* One relapsed and was given a second course of reserpine.

TABLE IVa.—*Response of Patients of Group 5—Acute Schizophrenia*

Much Improved	Improved	Relapsed	Untraced	Total
15*	2*	4	—	21

* One relapsed and was given a further course of reserpine.

It will be seen that the results confirm in a general way the trends of the initial assessment and encourage us to continue trial of this method of treatment.

A further year's experience suggests that smaller doses may give similar results with less distressing side-effects. At present an intramuscular dosage of 10 mg. daily is the exception rather than the rule. Our usual maximum dosage is 3 mg. orally and 5 mg. intramuscularly daily.

We hope to publish a further paper on a larger series with a detailed analysis of the factors influencing good or bad response, the relapse rate, and the incidence of depression.

REFERENCES

- Barsa, J. A., and Kline, N. S. (1955). *J. Amer. med. Ass.*, 158, 110.
 Doyle, A. E., and Smirk, F. H. (1955). *Practitioner*, 174, 135.
 Freis, E. D. (1954). *New Engl. J. Med.*, 251, 1006.
 Glynn, J. D. (1955). *J. Neurol. Neurosurg. Psychiat.*, 18, 225.
 Kline, N. S. (1954). *Ann. N.Y. Acad. Sci.*, 59, 107.
 — and Stanley, A. M. (1955). *Ibid.*, 85, 61.
 Noe, R. H., Williams, D. B., and Rapaport, W. (1955). *J. Amer. med. Ass.*, 158, 11.
 Plummer, A. J., Earl, A., Schneider, J. A., Trapold, J., and Barrett, W. (1954). *Ann. N.Y. Acad. Sci.*, 59, 8.
 Smirk, F. H., and McQueen, E. G. (1955). *Lancet*, 2, 115.
 Winsor, T. (1954). *Ann. N.Y. Acad. Sci.*, 59, 61.

IDIOPATHIC OR HYPERCATABOLIC HYPOPROTEINAEMIA

CASE EXAMINED BY ¹³¹I-LABELLED ALBUMIN

BY

MICHAEL SCHWARTZ, M.D.

Physician, Bispebjerg Hospital, Copenhagen

AND

BAGGER THOMSEN, Cand. Med.

Physician, Silkeborg County Hospital, Silkeborg, Denmark

Hypoproteinaemia is not an uncommon clinical finding, and is nearly always caused by an external loss of protein—for example, proteinuria, skin disease, bleeding—or a reduced protein synthesis (hepatic or intestinal disease, starvation, etc.).

In a few exceptional cases such low serum protein has been entirely inexplicable—idiopathic. A critical review of the literature shows that the reported cases which can be designated idiopathic hypoproteinaemia do not amount to more than 25.^{1 2 4 6-18 21 22} Despite this small number of cases the syndrome appears to be well defined.

The main sign of this disease is oedema formation caused by and varying with the hypoproteinaemia, which (nearly) always affects the albumin and globulin fractions equally. In most instances the disease has persisted for months and often for years. Practically all the reported cases of idiopathic hypoproteinaemia have been particularly well studied. In addition to the low serum

protein some of them have exhibited eosinophilia (4 out of 10 cases so examined) and several have been allergic, but all other tests have failed to show abnormalities.

Attempts at raising the serum protein level by oral administration of protein in large amounts have proved ineffective, although a highly positive nitrogen balance may be obtained thereby.⁷

Most authors have merely concluded that in idiopathic hypoproteinaemia protein synthesis is impaired. It has been suggested that this reduced protein synthesis is due to hepatic disease, but what kind of hepatic disease is not quite clear, as no patient with genuine idiopathic hypoproteinaemia has shown other signs of hepatic involvement.

The Degradation Theory

As no external loss of protein can be demonstrated and as there is no obvious cause why the production of protein should be reduced, the reason for the low serum protein might theoretically be imagined to be an internal loss—an increased degradation of protein.

Only a few authors have dwelt on this idea. As early as 1935, however Cope and Goadby⁷ stated: "The possibility that unduly rapid breakdown of plasma protein was occurring could not be excluded, but was considered to be improbable."

In recent years, however, reports on four patients with idiopathic hypoproteinaemia indicate that in these patients there has been a question of increased breakdown of protein. In 1949 Albright, Bartter, and Forbes¹ (1 patient) and in 1955 Hennemann and Albright¹¹ (2 patients) studied the fate of intravenously administered albumin. These extremely difficult and painstaking investigations will not be described in detail here: suffice it to mention that during the experiment the patients received 52 g. of serum albumin intravenously every day for more than two weeks.

The results of these balance experiments unequivocally showed that, compared with normal persons, the patients retained only a small proportion of the administered albumin during the experimental period, whereas an excessive amount must have been burned or converted into protoplasm. These authors conclude¹ that "the disorder in albumin metabolism was not a decreased rate of synthesis, but an increased rate of destruction."

Kinsell *et al.*¹⁴ studied the fate of intravenously administered ³⁵S-labelled methionine in some patients, including one with idiopathic hypoproteinaemia. This study also failed to show any signs of reduced plasma protein synthesis—rather the opposite. The rate at which ³⁵S disappeared from plasma protein by far exceeded that in normal people. Similarly, the urinary excretion of ³⁵S was increased compared with normal persons. Kinsell *et al.* sum up that "the basic defect is one of hypercatabolism."

Against the studies of Albright *et al.* it may be objected that intravenous administration of 50 g. of albumin daily for 16 days is rather an unphysiological procedure, and against the technique of Kinsell *et al.* that it may be difficult to assess the results of experiments with labelled amino-acids because of the possibility of their re-utilization. As patients with idiopathic hypoproteinaemia present special possibilities of elucidating the largely unknown mechanism which regulates serum proteins, it seemed to us of great interest to study the serum protein turnover, using directly labelled

protein. By this method only a few milligrams of protein are administered, and re-utilization is of no importance.

Such a study was carried out in the case reported below.

Case Report

A 25-year-old woman was first admitted to the Central Hospital, Silkeborg, on June 13, 1955, because of oedema of the legs. Her family history was negative. She had possibly had "jaundice" in childhood, but was otherwise in good health. Menstruation was normal. From the age of 17 she had been suffering from swelling of the ankles and lower legs, gradually ascending to the knees. The swelling had been most pronounced in the right foot and lower leg, but during recent years it had also involved the left hand. Facial oedema had never been observed. Apart from these swellings, there were no complaints. At another hospital the serum protein level was found to be 5.5% (albumin 3.7%, globulin 1.8%), when she was 20 years of age. At 22 she was treated in a surgical department for crural oedema by paste bandage. Phlebography as well as oscillometry showed normal findings.

The patient had spent a total of 113 days at the Central Hospital, Silkeborg, during which the condition had remained entirely unchanged, characterized by moderate but persistent oedema of the feet and lower legs. Upon bed rest they subsided slightly. Apart from the oedema nothing abnormal was found: development and nutrition were normal. Radiological examination of the stomach and small intestine gave normal findings. Chest radiography and E.C.G. were normal. X-ray films of sella turcica: small, measuring 10 by 7 mm. Ophthalmological examination normal.

Laboratory Tests.—Height, 154 cm.; weight, 48.9 kg. Haemoglobin, 90%; R.B.C., 4,560,000; colour index, 0.96; E.S.R., 1 and 2 mm./hour; W.B.C., 8,690 (staff cells 17%, polymorphonuclears 59%, eosinophils 8%, lymphocytes 13%, monocytes 3%). Sternal marrow: slight eosinophilia, no other definite abnormality. Urine: no albumin (several tests) or sugar. Blood urea: 20, 28, and 18 mg. per 100 ml.; blood urea clearance, 75% of normal. Serum bicarbonate, 27.6 mEq; serum chloride, 105–102 mEq; serum phosphorus, 6.6–3.8 mg. per 100 ml.; serum calcium, 9.3–8.6 mg. per 100 ml.; serum potassium, 16.7 mg. per 100 ml.; serum cholesterol, 122, 105, and 180 mg. per 100 ml.; total lipid in serum, 690 mg. per 100 ml.; alkaline phosphatases in serum, 2.5 units (Buch). Icterus index, 3. The bromsulphthalein, thymol turbidity, and Takata-Ara tests were negative. Faeces: No blood; excretion of fat normal. Adrenal cortical hormones in urine: 24-hour excretion of 17-ketosteroids, 6.2 mg.; reducing corticoids, 1.07 mg. (normal). Thorn's test with adrenaline, 50% decrease in eosinophils, with corticotrophin 25%, decrease (?). Skin tests with various allergens, all negative. Histamine test meal, free acid present. Glucose tolerance, normal curve. **Serum protein:** 4% (albumin 2.6%, globulin 1.4%). Paper electrophoresis: total protein 4.1% (albumin 53%, α_1 7.3%, α_2 13.6%, β 13.1%, γ 13.1%). Fibrinogen 650 mg. per 100 ml. (normal 200–400 mg.). Oedema fluid 0.2% (albumin).

Thus the essential result of the numerous laboratory tests was a marked decrease in serum proteins, a decrease which affected all fractions equally. In addition there was slight eosinophilia, whereas no other tests showed definitely abnormal values.

The patient had been tentatively treated with a 'high-protein diet for two weeks, with testosterone for three weeks, and with corticotrophin for 18 days without the slightest effect. During the observation period the serum protein values have remained about 4% and there have been no changes in the individual fractions.

Serum Albumin Turnover

For this study we used human serum albumin prepared at the State Serum Institute by the method of Cohn (more than

99% pure albumin), and labelled with ^{131}I by the method of Veall *et al.*²⁰ The finished iodinated albumin contains 5 atoms of iodine per molecule of protein; upon paper electrophoresis it is indistinguishable from non-iodinated albumin. The non-protein-bound radioactivity was <0.5%. Studies of the albumin turnover by ^{131}I -labelled albumin have been described on several occasions.^{5,19} The rationale of this method is that the labelled albumin will be dealt with by the organism just as its own albumin. When the labelled albumin has mixed with the subject's native albumin the plasma radioactivity will act as an indicator of the albumin's rate of degradation, which will also be reflected by the urinary excretion of ^{131}I .

Since the turnover rate of iodine-labelled proteins is more rapid than that of biologically labelled proteins, and since it may also vary within certain limits, presumably because, in spite of everything, proteins change slightly during iodination, the determination is relative rather than absolute. Each batch of labelled protein must therefore be tested on a number of normal persons in addition to the patients being investigated.

In our study the albumin turnover was determined in three normal persons as well as the hypoproteinaemic patient. All four subjects were hospitalized, given ordinary diet, and permitted moderate activity. In order to secure a rapid excretion of iodine and inhibit thyroid uptake of ^{131}I , we administered a 3% solution of potassium iodide, 30 g. daily.

The labelled albumin was injected intravenously (30 mg. of albumin with an amount of radioactivity of about 90 microcuries), and heparinized blood samples were drawn every day or every other day for more than two weeks, the first sample being obtained 10 minutes after the injection.

FIG. 1.—Plasma disappearance curve of ^{131}I -labelled albumin in two normal subjects.

FIG. 2.—Plasma disappearance curve of ^{131}I -labelled albumin in patient with idiopathic hypoproteinaemia.

The plasma radioactivity was determined on 0.4-ml. samples dried in aluminium dishes and counted with a Geiger counter and a Brüel and Kjær scaler. No correction was made for self-absorption (all specimens from the same subject were of the same weight).

The measurements of radioactivity (counts per minute) were corrected for decay if necessary, and converted to percentages of the 10-minute sample, which was also used to determine the plasma volume.

Fig. 1 gives the disappearance curve for the labelled albumin in a semilogarithmic scale for two of the three normal subjects, and Fig. 2 for the patient with idiopathic hypoproteinaemia. The curve consists of two parts: a curved portion extending over the first five to six days, corresponding to the mixing of the labelled albumin with the native albumin—that is, the “albumin phase”—and a straight line representing the degradation of the injected iodinated albumin (or the rate at which labelled albumin is replaced by unlabelled albumin).

The slope of the straight line indicates the rate of albumin turnover—the steeper the curve the quicker the turnover. The half-time ($T_{1/2}$) of the administered albumin may be determined graphically as seen from the figures, and from the equation representing exponential decay the decay constant (λ) may be calculated.

On the basis of the values shown on the curve and the serum albumin percentage it is now easy to compute the total amount of albumin in the body as well as its distribution into intravascular and extravascular sites (cf., for example, Sterling¹⁹).

The Table sets out these values for the three normal subjects and for the patient with idiopathic hypoproteinaemia.

Data Pertaining to Distribution and Degradation of ¹³¹I-labelled Serum Albumin in Three Normal Persons and Patient with Idiopathic Hypoproteinaemia

	Persons Studied			
	Normal			Idiopathic Hypoproteinaemia
	M.	F.	F.	
Age (years)	26	40	47	25
Weight (kg.)	83	70	53	50
Plasma volume (ml.) ..	3,970	3,460	2,780	2,200
Serum protein (%) ..	7.1	7.4	6.8	4.3
“ albumin (%) ..	5.47	5.03	4.50	3.01
Total exchangeable albumin (g.)	440	407	308	190
Intravascular albumin (g.) ..	217	174	125	65
Extravascular ..	223	233	183	125
Albumin turnover half-time ($T_{1/2}$) in days ..	21.3	22.7	23.9	7.2
Albumin degradation %/day ..	3.26	3.06	2.90	9.64
“ “ g./day ..	14.3	12.4	8.94	18.3
“ “ g./day kg. ..	0.172	0.177	0.169	0.366
Total renal excretion of injected dose ¹³¹ I in first 14 days following injection of labelled albumin ..			50%	91.9%

The result of the study is unequivocal. The patient with idiopathic hypoproteinaemia has a greatly increased albumin turnover. In our case the degradation rate for albumin was more than three times that in the normal subjects. Despite the greatly reduced amount of protein at her disposal the patient with idiopathic hypoproteinaemia degraded and synthesized over twice the amount of albumin per kilogram of body weight formed by normal persons. That the albumin is not merely removed, but also broken down, is shown from the fact that in our patient the renal excretion of iodine was about twice that in the normal subjects.

Discussion

The result—that idiopathic hypoproteinaemia is associated with marked protein catabolism—is in keeping with previous findings by Albright *et al.* as well as by Kinsell *et al.* The technique used by us cannot in any way affect the albumin metabolism as such. Only 30 mg. of albumin is administered, and the label substance (¹³¹I) is not re-utilized, but

excreted. The entire picture reminds one of that found by the same technique in nephrotic patients, apart from the fact that in the latter the lost protein is recovered in the urine.³ The old designation of idiopathic hypoproteinaemia “nephrosis without nephrosis” is in fact not out of place, although a linguistic monstrosity.

Now, three different methods of studying the protein turnover in a total of five patients with the rare disease idiopathic hypoproteinaemia have given the same result. It seems reasonable, therefore, to postulate that it is increased protein catabolism and not reduced synthesis which is the basal defect in all cases of this disease. A better name would therefore perhaps be “hypercatabolic hypoproteinaemia.” Still, this result does not solve the mystery of idiopathic hypoproteinaemia, but it must alter the approach of future studies. The mechanism which regulates the protein concentration of the blood is largely obscure. Perhaps the hormones of the adrenal cortex are operative, but we do not know how such regulation is effected. Messinger’s¹⁸ patient was apparently cured after having become pregnant, and Messinger interpreted this as a sign that the disease was due to temporary disturbance of anterior pituitary function (the patient had previously suffered from mild thyrotoxicosis).

A defect in the regulation of, at any rate, serum albumin may be seen from the fact that serum albumin remains low in these patients.

Balance at a low but not at a high albumin level must be interpreted on the supposition that the albumin level *per se* influences the catabolism, which must fall with a decreasing albumin concentration, or—presupposing a uniformly high albumin degradation regardless of the albumin concentration—that albumin synthesis increases with decreasing albumin concentration.

It is of particular interest that among the proteins of the body the plasma proteins appear to be affected selectively by the rapid degradation. The haemoglobin turnover, for instance, appears to take place at the usual rate, and experience from paediatric practice does not indicate a reduced rate of growth in children with idiopathic hypoproteinaemia. It must be mentioned also that these patients may well acquire a positive nitrogen balance. This would be difficult to explain if the protein catabolism in all tissues were increased to several times the normal.

Serum-protein hypercatabolism may remind one to a certain extent of the conditions in haemolytic anaemia, and this raises the question of how much serum protein the organism can produce. The present experiments determined only the albumin turnover. Kinsell *et al.* determined the total plasma protein turnover, so it is not yet known whether the rate of globulin turnover is increased as well. Since the present method utilizing ¹³¹I-labelled albumin must be presumed to afford a relative rather than an absolute measure of the albumin turnover rate, this question must await investigations using biologically labelled proteins.

Summary

Until recently it was believed that the low serum protein level in the rare disease idiopathic hypoproteinaemia was due to reduced protein synthesis.

Recent studies comprising a total of four patients indicate, however, that the basic defect is one of hypercatabolism.

A typical case of idiopathic hypoproteinaemia of eight years’ duration is reported. In this patient, too, a study of the albumin turnover with ¹³¹I-labelled serum albumin showed a greatly increased catabolism of albumin. We therefore suggest that the disease be called hypercatabolic hypoproteinaemia.

The investigation was aided by a grant from Statens Videnskabsfond, University of Copenhagen.

REFERENCES

- ¹ Albright, F., Bartter, F. C., and Forbes, A. P. (1949). *Trans. Ass. Amer. Phys.*, **62**, 204.
- ² Bálint, P. (1943). *Klin. Wschr.*, **22**, 540.
- ³ Bland, W. H., Fields, M., and Goldman, R. (1955). *J. Lab. clin. Med.*, **46**, 747.
- ⁴ Binger, M. W., and Keith, N. M. (1937). *J. Amer. med. Ass.*, **109**, 1.
- ⁵ Berson, S. A., Yalow, R. S., Schreiber, S. S., and Post, J. (1953). *J. clin. Invest.*, **32**, 746.
- ⁶ Bound, J. P., and Hackett, W. R. (1953). *Arch. Dis. Childh.*, **28**, 104.
- ⁷ Cope, C. L., and Goadby, H. K. (1935). *Lancet*, **1**, 1038.
- ⁸ Ehrengut, W. (1954). *Z. Kinderheilk.*, **74**, 141.
- ⁹ Forbes, A. P. (1944). In *Conference on Metabolic Aspects of Convalescence*, 6th meeting, p. 144. Josiah Macy jr. Foundation.
- ¹⁰ Hecht-Johansen, A. (1938). *Acta path. microbiol. scand.*, Suppl. **37**, p. 272.
- ¹¹ Henneman, P. H., and Albright, F. (1955). *J. Lab. clin. Med.*, **46**, 825. (Abstract.)
- ¹² Hertzog, F. W., and Faust, O. A. (1950). *J. Pediat.*, **36**, 641.
- ¹³ Homburger, F., and Petermann, M. L. (1949). *Blood*, **4**, 1085.
- ¹⁴ Kinsell, L. W., Margen, S., Tarver, H. McB., Frantz, J., Flanagan, E. K., Hutchin, M. E., Michaels, G. D., and McCallie, D. P. (1950). *J. clin. Invest.*, **29**, 238.
- ¹⁵ Messinger, M. J. (1946). *Canad. med. Ass. J.*, **55**, 381.
- ¹⁶ Meyer-Bisch, R. (1925). *Klin. Wschr.*, **4**, 588.
- ¹⁷ Rytand, D. A. (1942). *Arch. intern. Med.*, **69**, 251.
- ¹⁸ Salomonsen, L. (1945). *Nord. Med.*, **25**, 727.
- ¹⁹ Sterling, K. (1951). *J. clin. Invest.*, **30**, 1228.
- ²⁰ Veal, E. N., Pearson, J. D., Hanley, T., and Lowe, A. E. (1954). *Radioisotope Conference. 1954*, edited by J. E. Johnston, **1**, 183. Butterworth, London.
- ²¹ Wuhrmann, F., and Leuthardt, F. (1941). *Helv. med. Acta*, **7**, 565.
- ²² Wyngaarden, J. B., Crawford, J. D., Chamberlin, H. R., and Lever, W. F. (1952). *Pediatrics*, **9**, 729.

ORAL ADMINISTRATION OF PENICILLIN

WITH A NOTE ON METHODS OF ASSAY

BY

L. HENRY, M.B., Ch.B.
Senior House Officer

G. WHITE, M.B., Ch.B.
Senior House Officer

AND

M. J. MEYNELL, M.D., M.R.C.P.
Clinical Pathologist

*From the Department of Clinical Pathology,
General Hospital, Birmingham*

It has been noted that all preparations of penicillin, when given by mouth, are unstable in the acid medium of the stomach and are poorly absorbed from the gastro-intestinal tract. Oral penicillin therapy has thus been of value only in children, in whom the gastric acidity is lower (Cathie and MacFarlane, 1953).

In 1940 it was established that by the addition of various chemicals to the medium in which the mould was grown a variety of penicillins could be obtained, differing widely in their physical and antibacterial properties. Behrens *et al.* (1948), by using N-(2-hydroxyethyl)-phenoxyacetamide as the precursor substance, isolated phenoxyethyl penicillin. However, the *in vitro* effect of this differed only slightly from that of penicillin G, and it was not until Brandl *et al.* (1953) demonstrated its acid stability that its use as an oral penicillin was realized. Several articles appeared on the Continent confirming its value (Giovannini, 1954; Sjoberg *et al.*, 1955; Spitzzy, 1955), and the preparation was marketed under the name of "distaquaine V." The recent work in America (Jones and Finland, 1955; Martin *et al.*, 1955; Wright *et al.*, 1955) has been the subject of two leading articles (*B.M.J.*, 1956; *Lancet*, 1956), and we have already reported a preliminary survey of the serum levels obtainable (Henry and Meynell, 1956). Most papers have demonstrated that adequate and reliable absorption can be obtained with distaquaine V, but comparison is usually made with oral crystalline penicillin G. The latter is known to be

inadequately absorbed, the amount recoverable in the urine being in the region of 12% (Wright *et al.*, 1955). It was thus felt that injected penicillin would give a better basis for comparison.

The object of this paper is to compare the serum levels and urinary excretion rates obtained with phenoxymethyl penicillin (distaquaine V) and benzathine penicillin ("penidural") given orally, with crystalline penicillin G, and procaine penicillin ("avloprocil") given by intramuscular injection. In addition, the accuracy of the two standard methods of assay has been compared—the serial broth dilution method using *Staphylococcus aureus* (Oxford strain) and the plate method using *Sarcina lutea*.

Method

Six subjects were given 200,000 units of each preparation in turn, and thus acted as their own controls. The dose was given before meals and blood withdrawn at $\frac{1}{2}$, 1, 2, 3, and 4 hours, except in the case of procaine penicillin, when the intervals were $\frac{1}{2}$, 1, 2, 6, and 24 hours.

The bladder was emptied before the administration of the penicillin, and thereafter a 24-hour specimen of urine was collected; the volume was measured and an aliquot retained for assay. Serum levels were estimated on the same day by both methods, but in the case of the urine assays the *Staph. aureus* method was found to be unsuitable and the *Sarcina lutea* method only was used.

Staph. Aureus Method.—Serial dilutions of the serum are made in 1% glucose broth, containing Andrade's indicator, and to each tube is added one drop of an overnight culture of the Oxford strain of *Staph. aureus* (sensitive to 0.02 unit of penicillin per ml.). Readings are taken after 18 hours' incubation at 37° C. and the end-point is read according to colour change in the medium and visible growth, both being compared with a control estimation, using normal serum containing a known amount of penicillin. The end-point is sometimes difficult to judge, and it may be necessary to subculture the tubes on to a solid medium.

Sarcina Lutea Method.—Two media are used—a "base" layer with a "flood" layer superimposed. The base layer is made up of 1.5 g. of "lemco," 3 g. of "bacto" yeast, 6 g. of bacto peptone, and 15 g. of agar, suspended in 1 litre of water.

The flood layer is the same as the base layer with the addition of 1 g. of dextrose. The organism used is *Sarcina lutea* CPI—1001 (N.C.T.C. 8340). This is grown on slopes of flood layer agar, and after 18 hours' incubation is washed off with normal saline and the opacity is adjusted to that of a No. 8 Burroughs Wellcome opacity tube. If a standard Petri dish is used, base medium is poured in 10-ml. amounts and allowed to set. The flood medium is brought to 50° C. and is inoculated with the sarcina suspension, 2 ml. per 50 ml. of flood layer medium. Immediately after inoculation 4 ml. amounts of the medium are poured on to each plate containing the base medium. The plates are allowed to set and four holes punched in each, a No. 5 cork-borer being used, although the method is actually independent of the size of the hole.

Assay Procedure

Three plates are used for each assay. A standard solution of penicillin containing 0.1 unit per ml. is prepared, and the serum to be assayed is diluted to approximately the same strength with pH 7.5 phosphate buffer. Both these solutions are then diluted further (usually twice) with buffer, and four drops of a given dilution are introduced into the four holes on each plate, using a pipette delivering 70 drops per ml.

The plates are incubated at 26° C. for 18 hours, when the diameters of the clear areas are measured with a millimetre steel rule, and the average of the readings on the three plates is used in the calculation.