EFFECT OF INSULIN ON THE DUMPING SYNDROME

BY

C. T. HOWE, F.R.C.S.

J. CANDY, F.R.C.S.

L. P. LE QUESNE, D.M., F.R.C.S.

M. HOBSLEY, Ph.D., F.R.C.S.

AND

M. P. SPENCE, M.B., M.R.C.P.

From the Department of Surgical Studies, the Middlesex Hospital, London

The dumping syndrome is the commonest cause of distress after partial gastrectomy, and may also occur after total gastrectomy, after gastro-enterostomy, and after pyloroplasty. For many years it has been known that if patients with this syndrome drink a hypertonic solution of glucose their usual post-cibal symptoms will be provoked (Machella, 1949). It was shown by Roberts *et al.* (1954) that under these circumstances the symptoms are accompanied by a sharp fall in plasma volume, due, they suggested, to an osmotic transfer of fluid from the plasma into the gut lumen. They went on to suggest that after partial gastrectomy, with its removal of the pyloric control mechanisms, food in hypertonic solution passes rapidly into the intestine and causes similar effects, the ingress of fluid into the intestine being responsible for the abdominal component of the syndrome, while the fall in circulatory volume causes the systemic symptoms.

Since then it has been shown that there is not a close correlation between the occurrence of dumping symptoms and the size of the fall in plasma volume after the oral administration of hypertonic glucose solutions to patients who have undergone partial gastrectomy (Webber *et al.*, 1957). In a previous communication (Le Quesne *et al.*, 1960) evidence was brought forward suggesting that the occurrence of symptoms depends not only upon the size of the fall in plasma volume but also upon the individual patient's circulatory response to the fall. In another communication (Hobsley and Le Quesne, 1960) it was suggested that the size of the fall in plasma volume might ultimately depend on the rate of peripheral glucose utilization, and it was pointed out that if this theory were true then pre-cibal insulin in the appropriate dose might reduce the fall in plasma volume and modify the patients' post-cibal symptoms. Preliminary evidence has been published showing that insulin produces both these results, and the purpose of this paper is to report the results of further observations of the effect of insulin on the dumping syndrome.

Plasma-volume Studies

In a series of seven patients (three men, four women) with dumping symptoms the fall in plasma volume after a standard breakfast was measured, using the Evans-blue technique (Le Quesne *et al.*, 1960), and this observation was later repeated with a similar meal preceded by a suitably adjusted dose of insulin. On each occasion the test was performed between 8 and 9 a.m., with the patient sitting at a table, having taken nothing by mouth for the previous 12 hours. A precisely similar meal was

served to each patient. In five instances, at the first test the patients were persuaded and cajoled into eating as much as they possibly could, despite the onset of symptoms which would normally have caused them to stop eating: from the amount of food left their intake was then calculated, and at the second test they were given exactly what they had eaten on the first occasion. The other two patients were allowed to eat as much as they wanted on each occasion.

The results are set out in detail in Table I. In all seven patients the first meal provoked marked typical dumping symptoms, whereas after the second meal these symptoms were either absent or greatly reduced. In all seven patients the fall in plasma volume after the second meal was smaller than after the first ($t=7.95$; $P<0.001$). A typical result is shown in Fig. 1. Of the two patients allowed to eat as they wished at each meal, both definitely ate more at the second meal, and in both cases this second meal provoked no symptoms: in one case the second meal produced a distinctly smaller fall in plasma volume than the first, while in the second, though the fall was only slightly smaller, its duration was much shorter (Fig. 2).

One normal adult male was given 10 units of soluble insulin subcutaneously, with no ensuing meal. No change in plasma volume was detected, using the Evans-blue method, in the two hours following the injection.

Pre-cibal Insulin: Therapeutic Trial

Fifty-three patients with various post-gastrectomy syndromes (excluding anastomotic ulcer) were seen in

TABLE I.—Results in Seven Patients in Whom the Plasma Volume was Measured After a Meal Without and With Preceding Insulin

Case No.	Sex	Meal 1 (Without Insulin)		Meal 2 (Preceded by Insulin)		
		Fall in Plasma Volume (%)	CHO Intake (g.)	Fall in Plasma Volume (%)	CHO Intake (g.)	Dose of Insulin (units)
1	M	11	89	7	89	15
2	F	8	55	4	55	10
3	F	11	75	6	75	25
4	M	15.5	85	11	85	7
5	F	14	103	10	103	7
6	F	9	18	4	62	12
7	F	8	32	7	79	8

In all patients meal 1 was followed by marked dumping symptoms, whereas after meal 2 these were either absent or greatly diminished. The difference between the falls in plasma volume without and with insulin is significant ($P<0.001$).

FIG. 1.—Plasma-volume curves during the two test meals in Case 6. The first meal (top curve), containing only 18 g. of carbohydrate, produced marked symptoms (horizontal line) with a fall in plasma volume of 9%. After insulin he ate a meal containing 62 g. of carbohydrate with no symptoms and a fall in plasma volume of only 4%.

the four-year period ending February, 1960. Their symptoms were assessed clinically by one or more of us. In addition nearly all were given 175 ml. of 50% dextrose by mouth and their response was assessed (a) by noting the symptoms, if any, produced, and whether these resembled their post-cibal symptoms; and (b) by estimating the fall in plasma volume produced, either by direct measurement using the Evans-blue (T.1824) method, or by calculation from changes in the haematocrit and/or blood-glucose concentration (see Le Quesne *et al.* (1960) and Hobsley and Le Quesne (1960) for details of the methods involved).

As a result of this assessment it was decided that 18 of the patients either did not have the dumping syndrome or, if they did, that it was mild enough to

FIG. 2.—Plasma-volume curves during the two test meals in Case 7. The first meal (top curve), containing 32 g. of carbohydrate, produced marked symptoms with a fall in plasma volume of 8%. After insulin she ate a meal containing 79 g. of carbohydrate with no symptoms: the fall in plasma volume was 7%, but of much shorter duration than after the previous meal.

be controlled by simple dietary measures. Details of the remaining 35 patients, all of whom were treated with insulin, were as follows:

Twenty-one men, 14 women: average age 52.7 years (range 29–71 years): in 23 patients the original lesion was a duodenal ulcer, in 8 a gastric ulcer, in 2 combined duodenal and gastric ulcers, and in 2 carcinoma of stomach. The operation which these patients had undergone was in 20 instances a Polya-type partial gastrectomy, either retrocolic or antecolic, with a vagotomy in 3 cases; in 11 instances a Billroth I procedure; in 3 a gastro-enterostomy with vagotomy, and in 1 case a total gastrectomy with oesophago-jejunostomy.

Each patient was admitted to hospital for assessment, and to start treatment. Each was weighed by one of us on the same scales as were used thereafter in the follow-up clinic. In 10 instances no reliable information was obtained about the patients' change in weight since operation, but all of them had probably lost weight; two patients had gained slightly (4 lb. (1.8 kg.) each); the remaining 23 had lost weight, the average loss being 22.4 lb. (10.16 kg.) (range 7–56 lb.; 3.2–25.4 kg.). The average interval between operation and time of starting treatment was 51 months (range 8–156 months). In each case the patient's response to oral hypertonic glucose was noted; in all of them it reproduced their usual

post-cibal symptoms. In 34 patients the fall in plasma volume was either measured or calculated, the average fall being 12.3% (range 7–24%); in the remaining case this estimation was impossible for technical reasons.

In each case the dose of insulin (single strength, given subcutaneously) was found which would depress the patient's blood-sugar concentration, immediately before a meal, to the lowest level possible without producing symptoms due to hypoglycaemia. The initial dose was usually 10 units given 45 minutes before lunch. Guided by the patients' symptoms before and after the meal and by the blood-sugar concentration at the start of the meal, this dose was then altered until the optimal dose and time interval for each individual was discovered. Both these factors varied considerably from patient to patient, the average dose being 12.5 units (range 5–25 units) given on an average 50 minutes (range 20–75 minutes) before the meal.

Once the optimal dose had been discovered the patients were taught to give their own injection and then were allowed home to lead their ordinary life. At first most of them took insulin before their main meal only, but once they had gained confidence many of them took it twice daily. After leaving hospital all patients were seen in a special clinic, where their progress was followed, the treatment altered or abandoned as indicated, and their weight recorded.

To assess the results of this form of treatment, all those patients who had started insulin six months or more previously were placed in one of several categories, on the basis of their clinical response to treatment. This grading was performed by three of us, one of whom (M. P. S.) was in no way involved in previous work on this subject, and each patient was assigned to one of the categories shown in Table II.

TABLE II.—Categories Into Which Patients Were Graded, and Number Assigned to Each Group

Category	Description of Category	No. in Group
Group A: Patients still on insulin at time of assessment	A1 Definite benefit	8
	A2 Uncertain benefit	2
Group B: Patients no longer on insulin at time of assessment	B1 No benefit	3
	B2 Treatment abandoned owing to side-effects	2
	B3 Treatment beneficial, but abandoned owing to intercurrent illness	4
	B4 Undoubted benefit, but insufficient to warrant trouble of treatment	4
	B5 Permanent benefit, allowing cessation of treatment	6
	B6 Miscellaneous	4

Results

Two patients were lost to follow-up: it seems certain that neither is continuing with the treatment, but as assessment of their response is impossible they are excluded from this analysis. The grading of the remaining 33 patients is shown in Table II.

Ten patients were still on insulin at the time of assessment. Of these, eight have derived definite benefit, shown in part by an ability to eat more at meals preceded by insulin, with resultant gain in weight, and in part by a diminution or absence of symptoms after such meals. In these eight patients, of whom two are men and six women, the average maximum gain since the start of treatment is 9 lb. (4 kg.) (range 4–20 lb.; 1.8–9 kg.), and the average duration of treatment 24

(range 6-49) months. On one or more occasions each of these patients has tried the effect of stopping treatment, and all of them have asked to start treatment again because of a return of their previous post-cibal symptoms. A brief history of two of these patients illustrates their beneficial response to insulin treatment.

Case 24.—A foreman carpenter aged 56 years at start of treatment had undergone, 10 years previously, a vagotomy and posterior gastro-enterostomy for a chronic duodenal ulcer of many years' duration. Ever since the operation meals were followed by a feeling of severe abdominal distension and gurgling, sweating, palpitation, shakiness, and drowsiness, so that he had to lie down for 20 to 30 minutes. These symptoms restricted his food intake and he had lost 1½ stone (9.5 kg.) in weight. For his main meal he could take only a small helping of meat and vegetable, with no potatoes and no sweet. Oral hypertonic glucose reproduced his symptoms with a fall in plasma volume of 12.5%. In his case the optimal dose of insulin was 12 units 50 minutes before a meal, and since leaving hospital he has taken this before his midday and evening meals. At these meals he can eat distinctly more than previously and has no post-cibal symptoms, though at other meals the situation remains unchanged. A typical evening meal now is meat, potatoes, and green vegetable, followed by a sweet. He has occasional easily controlled "late" symptoms (see below). At the time of assessment he had gained 9 lb. (4 kg.) and had been on insulin for 18 months, during which period, for the first time since operation, he had eaten meals without discomfort.

Case 31.—A housewife aged 45 years at start of treatment had undergone partial gastrectomy for chronic gastric ulcer 14 months previously. Since then she had experienced typical severe dumping symptoms, with frequent post-cibal diarrhoea. The onset of symptoms restricted her main meal to one small course, and since operation she had lost 2½ stone (15.9 kg.) in weight. Oral hypertonic glucose reproduced her post-cibal symptoms, with a fall in plasma volume of 10.5%. She was found to be suited by 7 units of insulin 40 minutes before a meal. Since leaving hospital she has taken this before lunch and supper; at these meals she can now eat two courses, followed by a cup of tea with sugar, with no post-cibal symptoms and no diarrhoea. She has occasional easily controlled "late" symptoms after these meals; at meals not preceded by insulin the situation is as before. She has gained 11 lb. (5 kg.) in weight during 10 months on treatment.

In three patients (B1) the insulin treatment appeared to give no benefit. Four patients (B4) came to the conclusion that though the treatment was helping them the benefit was not sufficient to warrant the inconvenience of the injections and meals at a fixed time: of these patients, three gained weight during treatment, and all three promptly reverted to their previous weight on giving up insulin. In four patients (B3) the treatment, though apparently helping each of them, was abandoned owing to intercurrent disease which made control impossible: one patient developed fatal malignant hypertension, one had increasingly severe bronchitis and diverticulitis which required operation, and two developed severe depression. One of these latter, a middle-aged nun who had undergone Billroth I gastrectomy five years previously, was practically symptom-free on insulin and gained 15 lb. (6.8 kg.) in weight; her admission to a mental home led to cessation of the treatment, and she then began to lose weight again. In four instances (B6) treatment which was apparently helping the patients was abandoned for miscellaneous reasons unconnected with the patients' medical condition.

One of the difficulties encountered with this treatment is the liability to hypoglycaemic symptoms either just

before the meal or, more commonly, some 1½-2 hours afterwards. In most instances the symptoms were mild, the patients quickly learnt to control them by taking chocolate or a sweet drink at the appropriate time, and they rarely interfered seriously with the treatment. However, in two patients (B2) it was found that the dose of insulin required to give relief of the post-cibal symptoms caused such frequent and severe hypoglycaemic attacks that the treatment had to be abandoned. In one of these cases the dose of insulin in question was only 10 units.

There remain an interesting group of six patients (B5) who after a period on insulin appeared to be "cured." Initially they experienced benefit only after meals preceded by insulin. They then found that they were eating better, with fewer post-cibal symptoms, at other meals, and eventually that they could give up insulin without reverting to their previous condition. In these patients the time interval between operation and the start of treatment varied between 14 months and 9 years, the duration of treatment between 6 and 36 months. All these patients gained weight while on treatment (3½-14 lb.; 1.6-6.4 kg.). After stopping insulin one patient lost 1 lb. (0.45 kg.), two remained stationary. The following case history illustrates this type of response.

Case 7.—A housewife aged 47 years at start of treatment had undergone a Polya gastrectomy for chronic duodenal ulcer four years previously. After this she developed severe dumping symptoms, which had not improved with the passage of time, and which had led to a loss of 2 stone (12.7 kg.) in weight. On 10 units of insulin 45 minutes before lunch and supper she found that she could eat much more at these meals than previously with no post-cibal symptoms, and she gained 14 lb. (6.4 kg.). On several occasions she stopped insulin with a return of symptoms, but 36 months after starting treatment she found that she could give it up without deterioration, and this condition had been maintained three months later.

The results of the insulin treatment were analysed in relation to the duration of ulcer symptoms before operation, the length of time between operation and treatment, the type of ulcer, the type of operation, the loss of weight following operation, and the age of the patient: no correlation could be found between any of these factors and the results of insulin treatment. There did, however, appear to be a relationship between the patient's sex and the effect of treatment: only 2 of the 19 men showed definite benefit, compared with 6 of the 14 women.

Discussion

In a previous communication (Hobsley and Le Quesne, 1960) it was shown that after the oral administration of hypertonic glucose (175 ml. of 50% dextrose) to individuals who have undergone partial gastrectomy there is a direct relationship between the rate of rise of the blood-sugar concentration and the percentage fall in plasma volume, the more rapid the rate of rise of the blood-sugar concentration the bigger being the fall in plasma volume. This observation has since been confirmed (Castenfors, 1961). After removal of the pylorus, as in partial gastrectomy, the initial bolus of food or hypertonic glucose reaches the intestine more rapidly than usual, and hence initial absorption is faster than usual. In our previous paper (Hobsley and Le Quesne, 1960) it was, however, suggested that in those individuals with a rapid rise in blood-sugar concentration this rise reflects not so much a rapid absorption

as an inability of the tissues to remove the absorbed glucose fast enough to cope with this initial rapid rate of absorption. It was further suggested that this failure of glucose utilization to adapt to this challenge leads to a relatively slow absorption of glucose, which in turn results in a large osmotic shift of fluid into the gut, and thus a considerable fall in plasma volume: in contrast, in those patients with a slow rise in blood-glucose concentration, an adequate adaptation has developed, glucose absorption proceeds rapidly, with a smaller transfer of fluid into the gut and hence a smaller fall in plasma volume. Based on this hypothesis, the suggestion was put forward, supported by preliminary observations, that insulin, by increasing the rate of peripheral glucose utilization, might reduce the fall in plasma volume and lessen the individual's post-cibal dumping symptoms.

The results of this study show that an appropriate pre-cibal dose of insulin will lessen the fall of plasma volume that occurs during and after a meal in patients with the dumping syndrome, and that it will also, in many patients with this syndrome, diminish or entirely prevent their post-cibal symptoms, enabling them to eat more. These findings are of interest in relation both to the causation of the dumping syndrome and to its treatment.

It is possible that the effect of the pre-cibal insulin on the patient's post-cibal symptoms and food intake is entirely non-specific. For many years it has been known that small doses of insulin before meals will increase the appetite, and may in certain circumstances lead to an increase in weight. However, it seems unlikely that the results recorded here were due to such a non-specific effect, for the insulin not only improved or abolished the patient's symptoms but also reduced the fall in plasma volume occurring during and after a meal. Although other factors may be involved, there is good evidence that the temporary fall in circulatory volume occurring synchronously with the symptoms of the dumping syndrome is responsible for many, if not all, of the systemic symptoms of this condition (Le Quesne *et al.*, 1960). Thus, while it is possible that the effects of insulin on the patient's symptoms and on the size of the fall in plasma volume were unrelated, this seems unlikely. It seems more likely that the results observed were due to some specific effect of insulin, which by lessening the fall in plasma volume modified the patient's symptoms. Furthermore, the fact that insulin both reduces the fall in plasma volume and diminishes or abolishes the postprandial symptoms is further evidence that the symptoms are due to this fall.

As is shown by the experiment on the normal individual, insulin itself does not alter the plasma volume, and, as previously recorded (Hobsley and Le Quesne, 1960), the administration of insulin intravenously at the start of a meal does not influence dumping symptoms. It appears that insulin modifies the dumping syndrome only when it has had time to lower the blood-sugar concentration, so that its action in this condition is presumably through its effect on carbohydrate metabolism. It is possible that insulin hypoglycaemia achieves its effect in some way other than that suggested, though the observation that insulin lessens the fall in plasma volume lends support to the hypothesis, set out above, which led to its use. The observation does, however, strongly suggest that a disturbance of carbohydrate metabolism is intimately

concerned with the dumping syndrome, and this is further supported by the facility with which insulin produced hypoglycaemic symptoms in our patients. These symptoms most commonly occurred some 1½–2 hours after meals preceded by insulin, so that they developed some 2½–3 hours after an injection of insulin, despite an intervening meal, and, furthermore, they often followed a dose of insulin as small as 7–12 units. This is quite unlike the reaction of normal individuals to insulin, and, taken in conjunction with the effect of insulin on the fall in plasma volume, it suggests that not only carbohydrate absorption but also its metabolism is affected by operations which disrupt the normal mechanism controlling gastric emptying.

In assessing the efficacy of insulin in the treatment of the dumping syndrome, a gain in weight by the patient provides objective evidence of success: such a gain, up to a maximum of 20 lb. (9 kg.), was shown by many of our patients. A gain in weight is not, however, the only indication of benefit, for it is undoubtedly of benefit to a patient if treatment allows him to eat meals similar to those eaten previously but without post-cibal symptoms. Taking into account these two considerations, the majority of our patients derived benefit from insulin treatment, thus confirming the preliminary observations not only of ourselves but also of Glaessner (1940). However, in many the benefit was not great. It has previously been suggested (Le Quesne *et al.*, 1960) that the development of dumping symptoms depends not only on the fall in plasma volume but also on the individual's circulatory response to this fall. There may well be other factors involved as well. In such a complex situation it is not surprising that insulin, by altering one of these factors, does not give material benefit to all patients.

The treatment has the disadvantage that the patient must eat his meal at a fixed time after the injection. This causes inconvenience to many patients, and is probably the reason why the treatment was more often successful in women than in men, whose work, travel, etc., often made it difficult to take insulin at a fixed time before a meal. Further, the treatment requires intelligent co-operation on the part of the patient. For these reasons, and also in view of the small but definite incidence of hypoglycaemic attacks both before and after meals, it is clear that insulin has only a limited place in the treatment of the dumping syndrome, though it appears to be of considerable benefit to a number of patients. It is possible that the oral hypoglycaemic drugs may be of more value, as suggested by Nabarro (1962), in view of their slower effect on the blood-sugar level, and initial trials with tolbutamide are encouraging.

Six patients who derived benefit from insulin were able, after a while, to give it up without experiencing a return of their previous symptoms. It may well be that with the passage of time their symptoms will return, but at the time of assessment it appeared that a period on insulin had produced a definite change, and it is only possible to put forward tentative explanations to account for this. In the early weeks after partial gastrectomy many patients experience quite severe dumping symptoms, and in the majority these symptoms improve markedly in two to three months. The mechanism of this spontaneous remission is unknown, but on the basis of the hypothesis set out above it might lie in an alteration in their carbohydrate metabolism. It seems possible that in a certain number of patients in whom

this alteration has not occurred spontaneously a period on insulin treatment might induce the requisite adaptation.

Summary

In seven patients with the post-gastrectomy dumping syndrome a pre-cibal injection of insulin was shown to reduce the post-cibal fall in plasma volume, at the same time relieving their symptoms.

Thirty-three patients with the dumping syndrome were treated with insulin before one or two meals a day. All but three showed some benefit, though in only 14 was this definite: of these, six were able, after a while, to give up treatment without relapse.

The possible mechanism by which insulin achieves these effects is discussed. In patients with the dumping

syndrome it appears that their carbohydrate metabolism has failed to adapt to the new circumstances produced by the operation.

It is concluded that insulin treatment is of benefit in some patients with the dumping syndrome.

REFERENCES

- Castenfors, M. L. (1961). *Scand. J. clin. Lab. Invest.*, **13**, Suppl. 62.
 Glaessner, C. L. (1940). *Rev. Gastroent.*, **7**, 528.
 Hobsley, M., and Le Quesne, L. P. (1960). *Brit. med. J.*, **1**, 147.
 Le Quesne, L. P., Hobsley, M., and Hand, B. H. (1960). *Ibid.*, **1**, 141.
 Machella, T. E. (1949). *Ann. Surg.*, **130**, 145.
 Nabarro, J. D. N. (1962). In press.
 Roberts, K. E., Randall, H. T., Farr, H. W., Kidwell, A. P., McNeer, G. P., and Pack, G. T. (1954). *Ann. Surg.*, **140**, 631.
 Webber, B. M., Bender, M. A., and Moore, G. E. (1957). *New Engl. med. J.*, **256**, 285.

BLOOD GROUPS AND ENZYMES OF HUMAN RED CELLS AFTER A YEAR'S STORAGE IN LIQUID NITROGEN

BY

R. G. HUNTSMAN, M.B., B.Chir., M.R.C.P.

Chemical Pathologist, Memorial Hospital, Peterborough, Northants

ELIZABETH W. IKIN, B.Sc.

Senior Scientific Officer, Medical Research Council, Blood Group Reference Laboratory, Lister Institute, London

B. A. L. HURN, M.B., B.S.

Registrar, Department of Pathology, Lewisham Hospital, London

H. LEHMANN, M.D., Sc.D., M.R.C.P., F.R.I.C.

Reader in Chemical Pathology, St. Bartholomew's Hospital, London

J. LIDDELL, M.A., M.D.

Senior Registrar, Nuffield Department of Clinical Biochemistry, Radcliffe Infirmary, Oxford

The identification of blood-group antibodies in human sera is usually performed in specialized laboratories. Much of this work could be done in routine laboratories were it not for the practical difficulty of keeping the necessary panel of red cells of known antigen constitution. At present they are preserved in glycerol at about -15 to -20° C. (or very occasionally at -40° C.), a technique introduced by Smith (1950) and developed by Crawford, Cutbush, and Mollison (1954). Glycerol-preserved cells cannot be stored in an ordinary deep-freeze for much more than one year because of progressive haemolysis. Moreover, the thawing of the cells and removal of the glycerol is slow and tedious.

An alternative procedure is the storage of red cells in liquid nitrogen. In this technique the freezing and thawing is rapid enough to avoid gross injury to the red cell. Additional protection is given if suitable sugars are added to the blood before freezing (Meryman and Kafig, 1955; Huntsman, Hurn, and Lehmann, 1960; Strumia, Colwell, and Strumia, 1960). These substances, unlike glycerol, are easy to remove after thawing. Originally the technique was developed for the preservation of blood for transfusion, but many of the practical difficulties are still unsolved. This aspect of work on storage in liquid nitrogen has been reviewed recently by Lehmann and Hurn (1961) and Hurn, Huntsman, and Lehmann (1962). Huntsman *et al.* (1960, 1962) suggested that the simplicity of the method made it highly suitable for serological work and showed that it had no adverse effect on the range of red-cell antigens tested. Bronson and McGinniss (1962) reported good preservation of agglutinogens after six months' storage.

The present paper provides a practical system for the freezing, storing, and recovery of red cells and shows

the effect of one year's storage on the antigenic structure and percentage haemolysis. The biochemical integrity of the red cells has also been investigated by the serial estimation of four intracellular enzymes over the same period.

Apparatus

Cabinet for Liquid-nitrogen Storage.—Type L.N.R. 25 B, made by the Linde Company (a branch of Union Carbide Co.), is supplied with six canisters (Fig. 1) which require further modification (Figs. 2 and 3). The cells are kept in cardboard boxes ("corruganza" seamless ointment boxes, new No. 20 size), eight of which can be kept in each canister (Fig. 3). They are labelled as required on their lids and sides with a grease pencil. To label a box while it is cold, a "magic marker" should be used. The canisters should be kept full of boxes, even if some of the latter are empty. The cabinet needs replenishing with liquid nitrogen at intervals of about one to two weeks. If no reserve supply of liquid nitrogen is kept in a separate Dewar flask a simple ladle for removing small amounts of nitrogen from the main storage cabinet will be required. A thin-walled long-handled ladle is most suitable. This is manufactured by welding a bottom and handle to a metal cylinder 5 cm. in diameter and 10 cm. in length.

Method

Preparation of Blood.—Whole blood was treated with anticoagulant by the addition of 1 mg. of ethylenediamine tetra-acetic acid (E.D.T.A.) per each ml. The sodium, potassium, or lithium salt of E.D.T.A. is equally satisfactory. The blood should be frozen within one or two days of collection. Not longer than two hours before freezing, the blood is mixed with half its volume