Variability of Blood Sugar Levels With an Automated Method

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RESULTS of blood glucose determinations vary considerably from one laboratory to another (*1*). Variations are caused not only by the many different methods used but also by factors influencing the work of individual technicians. Pipetting differences, imperfect calibration of pipettes, inconsistent mixing of solutions, or inadequate control of temperature during reaction can cause generally reliable technicians within a given laboratory to obtain different mean results even when given aliquots of the same sample. Physical and emotional distractions also can affect the performance of otherwise efficient personnel.

Automation would seem to assure both greater uniformity and increased speed for such biochemical procedures. Several commercial methods are now available, but because results may also vary among the types of automated procedures, each process must be individually appraised.

We confined our study to one of the more widely used machines, the AutoAnalyzer (*2*), and to its manufacturer's recommended methods for blood sugar estimation. Our aim was to achieve a realistic appraisal of its precision and some factors which might affect interpretation of its results when used for clinical or research purposes. Since the AutoAnalyzer is capable of processing large numbers of specimens on a continuous basis, its evident value in public health epidemiologic and screening programs makes determination of its precision a desirable prerequisite.

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Methods

Glucose determinations in this study were performed on the AutoAnalyzer (*A*) at a sampling rate of 40 per hour. The procedure was that recommended by the company, being a modification of the ferricyanide method of Hoffman (*3*). Except where indicated, the macro method was used. Blood samples were collected in vacutainer tubes (*B*) and either used as such or centrifuged for plasma as required. For serum determinations, blood collected in plain tubes was allowed to clot and the serum separated within half an hour after clotting and refrigerated.

Four glucose recovery studies were performed within a period of 2 months. The first two studies used commercial bovine serum; for the third and the fourth, single donors gave blood samples the evening before the determinations were performed.

Two duplicate recovery studies were performed by the micro method on the whole blood samples.

Results

Recovery studies. The AutoAnalyzer showed consistently good recovery of glucose in all four studies ranging from 96.5 to 100.9 percent. Even the micro method yielded good recoveries ranging from 95.6 to 101.1 percent (table 1). In general, the higher levels of blood sugar were

accompanied by decreased precision. Use of the micro method proved unsatisfactory as indicated by the higher standard deviations about these mean results.

Reproducibility. The method showed excellent reproducibility for consecutive determinations on the same sample (table 2). The favorable reproducibility indicated by consecutive determinations of aliquots of the same sample (for example, mean whole blood 89.7± one standard deviation 0.89 mg./100 ml.) gives confidence only to the glucose recovery studies and does not accurately reflect the precision of the method used daily during a period of time. A pool of strained hemolyzed blood was divided into 141 aliquots and stored at −20° C. As part of a quality control program, one aliquot was included with each daily set of determinations for 2 months. The resulting mean of 129.9 mg./100 ml. with one standard deviation

Table 1. Glucose recovery studies

Sample and number of determinations	Glucose added (mg./100 ml.)	Glucose expected (mg./100 ml.)	Glucose recovered mean±1 S.D. (mg./100 ml.)	Percent recovered
AutoAnalyzer (Hoffman ferricyanide method)				
Serum A:				
6			84.3±0.8	
6	93.6	177.9	176.0±1.4	98.5
6	107.4	191.7	190.5±1.2	99.4
Serum B:				
10			83.3±0.9	
10	75.0	158.3	159.8±0.7	100.9
10	132.6	215.9	216.4±0.7	100.2
Whole blood A:				
10			89.7±0.9	
10	31.8	121.5	117.2±1.6	96.5
10	65.2	154.9	154.1±2.4	99.5
Whole blood B:				
10			73.6±0.8	
10	20.0	93.6	91.6±0.9	97.9
10	71.0	144.6	139.8±1.4	96.7
10	94.2	167.8	163.7±2.8	97.6
Micro method				
Whole blood A:				
10			86.2±6.7	
10	31.8	121.5	119.9±6.4	98.7
10	65.2	154.9	155.4±11.4	100.3
Whole blood B:				
10			71.8±5.6	
10	20.0	93.6	90.9±3.9	97.1
10	71.0	144.6	138.3±6.2	95.6
10	94.2	167.8	169.7±8.9	101.1

Table 2. Repeated determinations on samples of various composition

Sample	Known glucose value (mg./100 ml.)	Number of determinations	Mean AutoAnalyzer result (mg./100 ml.)	One standard deviation (mg./100 ml.)
Glucose (aqueous solution)	125.0	40	[1] 126.1	±2.5
6 percent bovine albumin	125.0	42	[2] 129.1	±3.5
Serum pool [3]	150.0	117	151.7	±4.1

[1] Difference statistically significant (P=.05).
[2] Difference statistically significant (P=.01).
[3] Glucose destroyed by incubation; then weighed glucose added.

range of ±4.6 mg./100 ml. indicates the greater variability over time. Similarly, a human serum pool, prepared by incubation removal of glucose with reconstitution to 150 mg./100 ml., following passage through a bacterial filter was found to have a mean of 151.7 mg./100 ml. with a one standard deviation range of ±4.1 mg./100 ml. (table 2).

Sample content. In addition to illustrating the greater variability of the procedure over time as compared with a single day, the serum pool is one of three pools of various compositions with known glucose concentrations used in testing a quality control program. The results are tabulated (table 2). Since the glucose content of the samples is known, it can be seen that the nonglucose constituents affect the result.

The observation that protein (*2*), by its effect on dialysis, will increase the resulting blood sugar level is also suggested by these results. In addition, it might be noted that the expression of reproducibility by the standard deviation about the mean does not given any indication of the accuracy of the method, which is related to the true value.

Machine "noise." Continuous running of a standard glucose solution containing 150 mg./100 ml. illustrates the amount of variation attributable to the "noise" of the machine. Problems with the manifold, dialyzer, or bubble pattern can increase this beyond acceptable limits (± 0.5 percent transmission line), and a variation greater than 3 mg./100 ml. can and does result. Unacceptable noise limits occasionally disappear spontaneously without a specific cause being identified.

Reproducibility of the standard curve. Since the standard curve is generally found to be reproducible, its minor variations are usually disregarded. Both known and unknown samples, having been handled identically by the same tubing, are commonly assumed to vary to the same degree, thus producing no appreciable difference in results. The fact that this is not always true is illustrated in the chart in which two sets of determinations indicate a disproportionate relationship between the standard curve and the control sample. The sample was prepared to contain 150 mg./100 ml. but read 151 mg./100 ml. from the first and 144 mg./100 ml. from the second curve. This was examined further by placing a 125 mg./100 ml. aqueous standard among the unknowns and judging how closely it followed its fellow aqueous standards in the calibration curve. Five series on the same day, including a total of nine determinations, gave a mean of 124.3 mg./100 ml. with one standard deviation of ±2.0 mg./100 ml. The same aqueous standard run over a period of time, for a total of 40 determinations, gave a mean of 126.1 mg./100 ml. with one standard deviation of 2.5 mg./100 ml.

The imperfect reproducibility indicated by these results probably has multiple causes. One of these is the stretching by the proportioning pump of the tubings of unequal sizes and unequally affecting their bores. This would change the proportions of the substances passing through and thus contribute to the varying results.

Sample arrangement. The reproducibility of the method is in part dependent upon the arrangement of the specimens in the sample plate, that is, the order in which they are run. Aliquots from a serum pool were run in pairs. The first of the pair was constantly positioned following the standard curve and water, while the second was placed among the unknown blood samples. Analysis was made following 30 daily determinations. The mean for the sample following the water passage was 149.8 with one standard deviation ±2.6 mg./100 ml., while for the sample among the unknown blood specimens it was 151.5 with one standard deviation ±2.9 mg./100 ml. The higher mean (statistically significant, $P<.01$) and greater variability of the second of the pair is in part due to contamination by the previous sample. In general, the greater the difference between the blood sugar concentration of the two specimens, the greater is the effect. Other factors are concerned, however, as indicated by the variability of the first of the pair which was estimated only when water had cleared the system.

Handling of sample. Sedimentation of whole blood on the sample plate will result in proportionately more cells being aspirated and thereby contribute a lower blood sugar result. Similarly, persons with a high hematocrit will have comparatively low blood glucose values by this method and vice versa. This effect was tested with fresh blood from four donors, two

Disproportion between serum control and standard curves

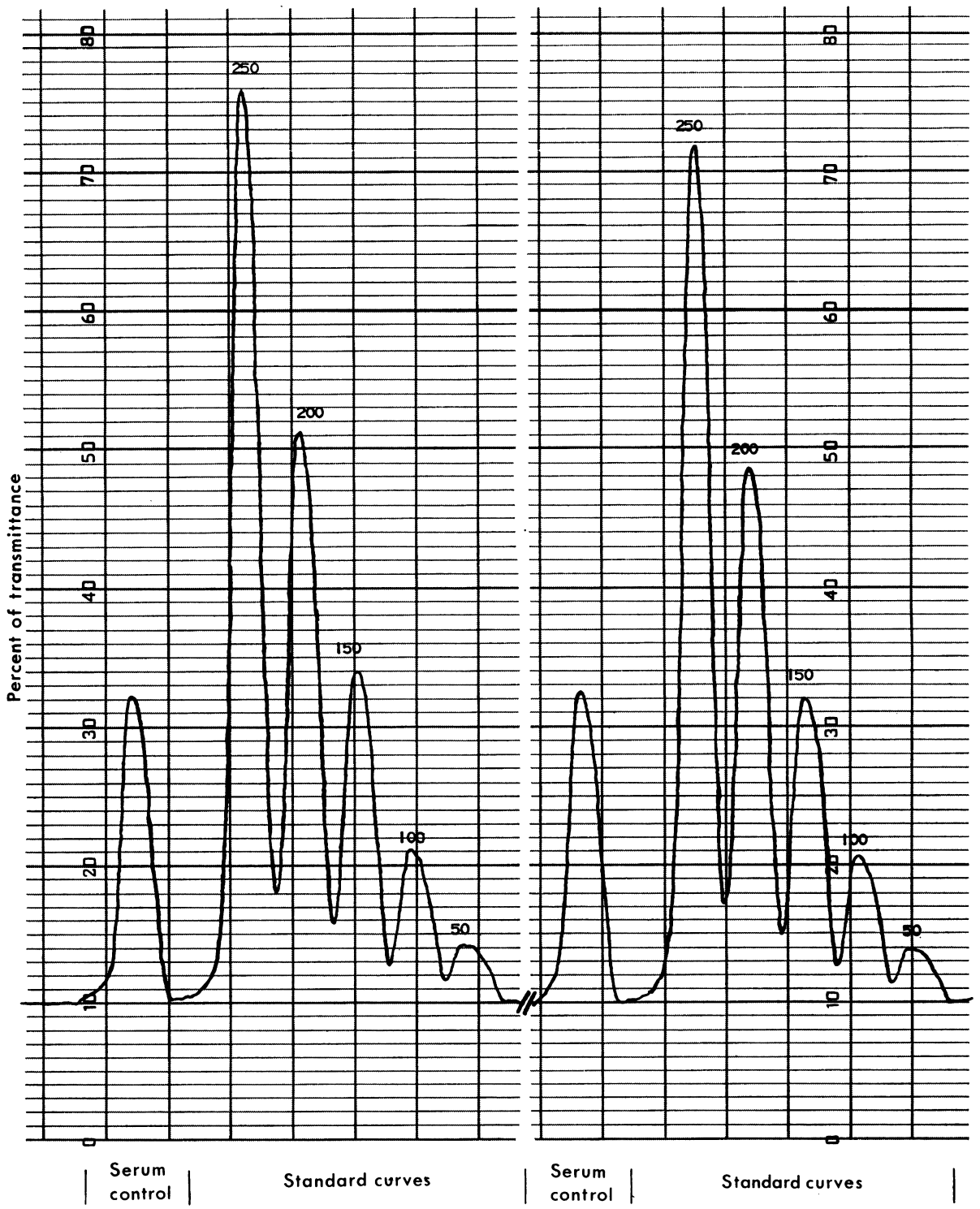

Table 3. Effect of hematocrit on blood sugar determinations

Original blood (glucose concentration in mg./100 ml.)	Blood with raised hematocrits (glucose concentration in mg./100 ml.)	Blood with lowered hematocrits (glucose concentration in mg./100 ml.)
90	82	96
58	56	64
93	81	101
64	60	69

male and two female. Two vacutainers of blood were obtained from each person. After the blood sugar was determined on tube number 1, tube number 2 was centrifuged and 2 ml. of plasma removed and added to tube number 1. Thus, there were samples of regular blood, blood with raised hematocrits, and blood with lowered hematocrits. The means of the four determinations of each group are shown in table 3.

The 2.5 percent evaporation seen to occur in 1 hour in our laboratory tends conversely to increase the values. Hemolysis by its release of protein and saccharoids will also raise the resulting value. This effect was quantitated roughly by comparing the glucose content of 75 whole blood samples before and after the hemolysis produced by freezing and thawing. The mean differences of the hemolyzed samples were higher by 5.4 mg./100 ml. with one standard deviation ±3.3 mg./100 ml. Incomplete anticoagulation may result in small clots interrupting the flow of the samples in the tubing or coils, having an obvious effect on the results. The volume of the sample will also be variously affected by the level of fluid in the sample cup since the sample tube is continuously sucking whether it is dipped into the specimen or not, so that more will be aspirated from a full cup than from one half-empty.

Miscellaneous factors. Many other factors appear critical in obtaining good precision with the AutoAnalyzer. The constant temperature bath will occasionally develop problems with marked effects on the final determinations. The cleanliness of the cuvette and the degree of use of the membranes are very important. The speed of sampling is inversely related to the precision of the instrument. Of the three possible speeds, 20, 40, and 60 per hour, the middle one is recommended as a suitable compromise when the 6-mm. cuvette is used. The flow cuvette is available in several thicknesses. The greater the thickness, the greater the absorbance, and the more sensitive is the test. However, the washout in the bigger cuvettes is less complete, which makes the final reading more sensitive to the volume of the sample and the concentration of the previous specimen.

Comparative levels with clinical samples. Venous blood samples obtained from 161 patients were analyzed for whole blood and plasma glucose. Within this group, 45 patients had their serum glucose analyzed. Fewer serum determinations were obtained since the absence of fibrinogen which distinguishes serum from plasma was not expected to have any material effect on dialysis. The results indicated no statistical difference between the means for plasma and serum. Glucose values in plasma, however, were higher than those in whole blood by a mean of 16.0 mg./100 ml.

Table 4. Comparative glucose values for whole blood and plasma

Level of glucose in whole blood (mg./100 ml.)	Number of samples	Mean difference between whole blood and plasma (mg./100 ml.)	One standard deviation (mg./100 ml.)	Percent difference between plasma and whole blood
116.0 (mean)	160	15. 6	5. 5	13. 4
100	65	10. 6	4. 8	12. 6
100–129	40	[1] 15. 8	6. 9	13. 8
130–159	38	[1] 21. 2	7. 8	14. 9
160	17	21. 9	7. 2	12. 3

[1] Significantly different from preceding mean (P=.01).

The range of differences, while quite large, was examined by increasing levels in whole blood. The results were tabulated (table 4). In general, plasma glucose was found to be approximately 14 percent higher than the corresponding whole blood level. The analysis also included testing which revealed no significant contribution to these reported differences from such influencing factors as hematocrit and the partial hemolysis found in some of the specimens. Hemolysis was checked by hemoglobin determinations on the plasma. The mean differences of all specimens with plasma hemoglobins of less than 1 gram was not statistically different from the mean differences of the whole group.

Discussion

Experience in our laboratory over a period of years indicates that results obtained with fresh venous whole blood on the AutoAnalyzer are approximately 2 to 3 mg./100 ml. higher than those obtained with the manual Somogyi-Nelson method. This is surprising since the ferricyanide method is relatively nonspecific. The unruptured red cells holding the saccharoids, in addition to their volume displacement of plasma, are probably the chief factors operating to maintain this proximity. Many of the problems with the conventional manual methods are associated with the varied effects produced by different methods of protein precipitation. Since the AutoAnalyzer utilizes the principle of dialysis this problem is bypassed, but by so doing it creates a series of problems which do not arise with the standard manual procedures. Typical difficulties include the effect on the resulting glucose value of varying degrees of hemolysis or the protein content of the sample.

Many of the difficulties described can be corrected by careful attention to detail and good planning. Results that can be interpreted with some confidence will then be obtained. If the use of whole blood is contemplated, added care in avoiding hemolysis during and after the venipuncture is required. In addition, it must be remembered that hemolysis will increase with the length of time the specimen is stored. Freezing the sample will affect the result by the same mechanism. Inadequate anticoagulant may give rise to the small clots which affect the flow of the sample in the machine's tubing. Use of serum will avoid many of the problems in this category, although there is the added inconvenience of having to separate it from its clot as soon as possible to avoid the resulting progressive loss of glucose by glycolysis. On the other hand, results with plasma samples may be affected by contamination from hemolysis, small clots, or inadequate mixing if there is layering following storage by freezing.

Precautions can also be taken with the machine when suitable samples have been obtained. It is recommended that a preliminary daily check of the "noise" pattern be made by aspirating a 150 mg./100 ml. glucose standard for about 5 minutes. If the resulting pattern is greater than ± 0.5 transmission line, the various sections of the machine, such as the manifold. dialyzer, and amplifier, should be checked. Examining each sample for clots is also advisable. The use of a bubbler (*4*) avoids layering in serum and sedimentation of cells in whole blood. Unfortunately, the bubbler may lead to foaming, which, if corrected by the addition of caprylic alcohol, may alter the resulting glucose value by causing hemolysis. The recent development of a mechanical stirrer would appear to be a better solution than the bubbler. Covering the specimens will reduce the evaporation from 2.5 to 0.5 percent per hour. Filling the sample cups to the same level as the cups containing the standards is also desirable. Following the provided maintenance chart consistently will prevent many other problems.

The factors affecting glucose values from capillary samples are greater than with venous blood samples both during collection and storage. The much increased variability of the results with the micro method on the AutoAnalyzer is disappointing. This is particularly true when it is considered that our results were obtained under ideal conditions, with use of venous blood and avoidance of many problems encountered during the routine collection of capillary specimens.

In conclusion it might be said that the variability in blood sugar determinations by this procedure is greater than is generally realized. The use of automation gives a false sense of

security. Some of the difficulties contributing to this lack of precision can be controlled. In such circumstances results will be more consistent than those obtained in a routine clinical laboratory. Knowledgeable and alert supervision is required at all times, and careful handling of specimens becomes of greater importance.

Summary

Four glucose recovery studies with both serum and whole blood were made by the Hoffman ferricyanide method on the AutoAnalyzer. Consistently good recovery of glucose was demonstrated. Two duplicate studies using the micro method indicated excessive variability.

No difference was found between serum and plasma glucose levels. Plasma levels were higher than those in fresh whole blood by a mean of 16.0 mg./100 ml. with one standard deviation ±9.3 mg./100 ml. When examined by level of whole blood, the plasma was found to be approximately 14 percent higher.

Reproducibility was investigated by repeated determinations over time on a hemolyzed blood pool which had a mean of 129.9 mg./100 ml. and one standard deviation of 4.6 mg./100 ml. Similarly, 117 determinations on a human serum pool, whose glucose was destroyed and reconstituted to 150 mg./100 ml., were found to have a mean of 151.7 mg./100 ml. with one standard deviation range of 4.1 mg/100 ml.

Some factors contributing to precision were considered. The variation in the standard curve was not always paralleled by an equivalent variation in the unknown sample. The condition of the sample had an effect on results by this method where none would have been expected with conventional methods. Hemolysis, decreased hematocrit, and increased protein content all increase the resulting glucose value. The effects peculiar to this method are generally caused by factors influencing dialysis. In addition machine problems arise. Small clots may block the tubing. Mechanical failure or poor maintenance can have a more obvious effect.

Knowledgeable and alert supervision is required at all times if results which can be interpreted with confidence are to be obtained from the AutoAnalyzer. Under these circumstances results will be more consistent than those obtained in a routine clinical laboratory.

REFERENCES

(*1*) Beck, W. P., and Sunderman, F. W.: A survey of the accuracy of chemical analysis in clinical laboratories. Amer J Clin Path 17: 853–861 (1947).

(*2*) Skeggs, L. T.: An automatic method for colorimetric analysis. Amer J Clin Path 28: 311–322 (1957).

(*3*) Hoffman, W. S.: A rapid photoelectric method for the determination of glucose in blood and urine. J Biol Chem 120: 51–55 (1937).

(*4*) Naumann, H. N., Olsen, A. M., and Young, J. M.: Bubble mixing device for blood sampling by automatic analysis. Clin Chem 7: 70–74 (1961).

EQUIPMENT REFERENCES

(*A*) AutoAnalyzer, Technicon Instruments Corporation.

(*B*) Vacutainers. No. 3204X, Formula 44, and No. 3204X, Formula 91, Becton Dickinson Company.

Conference Calendar

January 25, 1964: Industrial Hygiene and Air Pollution, University of Texas, Austin. Joe O. Ledbetter, 305 Engineering Laboratories Building, University of Texas, Austin, Tex., 78712.

January 23–24, 1964: Industrial Water and Waste, University of Texas, Austin. Dr. J. F. Malina, Jr., General Chairman, Engineering Laboratories Building, University of Texas, Austin, Tex., 78712.

March 1–6, 1964: American College of Allergists (graduate instructional course and twentieth annual congress). The Americana, Bal Harbour, Miami Beach, Fla. John D. Gillaspie, M.D., Treasurer, 2141 14th St., Boulder, Colo.

December 2–4, 1963: Solid Wastes Research Conference, University of Chicago Center for Continuing Education. Dr. Ross McKinney, Chairman, Civil Engineering Department, University of Kansas, Lawrence, Kans.

Announcements of meetings of national interest to public health should be forwarded to Public Health Reports 6 months in advance.

Food for Peace

Food for Peace is the name given to programs using surplus U.S. farm products abroad as authorized in 1954 by Public Law 480, the Agricultural Trade Development and Assistance Act. The programs range from feeding the hungry to long-range economic development. Farm products worth more than $12 billion have been shipped to 134 countries and territories.

The programs are supervised and coordinated by the Director of Food for Peace, who is part of the White House staff. The U.S. Department of Agriculture, through the Commodity Credit Corporation, determines the kinds and amounts of foods and fibers available for the programs and supervises the processing of the food and transportation to the port of embarkation. The products shipped include wheat and flour, feed grains, rice, cotton, tobacco, dairy products, fats and oils, poultry, beans and peas, and fruits and vegetables. The Department of Agriculture also carries out the sales and barter agreements of the act. The Agency for International Development administers the overseas operations, including integration of food aid with the rest of the economic assistance extended to underdeveloped areas.

Public Law 480 has four sections, or titles. Under title 1, foreign countries may buy U.S. farm products with their own currencies. The foreign currencies derived from these sales are used for payment of overseas costs of American embassies and other government programs, loans and grants to foreign countries for economic development, and other purposes. As of July 1962, title 1 grant and loan agreements totaled $5.4 billion, nearly 64 percent of the total for Food for Peace programs.

India has purchased title 1 commodities worth more than $2 billion. By drawing on the "food bank" of U.S. farm products, India has been able to avoid famine and an inflation that could cripple its development program. More than 80 percent of the rupees from title 1 sales has been set aside as loans and grants for projects in education, highways, electric power, and malaria eradication.

Title 2 authorizes the use of food as part payment of wages for work in school construction, reforestation, highway repair, and other projects designed to encourage economic development. It also permits emergency provision of food for disaster areas and for refugee relief, for child feeding programs, and for colonization projects.

To aid drought-stricken northeastern Brazil, the United States and Brazil signed a title 2 agreement in 1962 that provided 26,000 tons of food valued at $5.4 million. The food is being used as part payment of wages for work on projects planned by the Brazilian government, with the result that hunger is being alleviated and permanent improvements carried out in the communities.

Under title 3, food is donated for the overseas programs of voluntary organizations such as CARE, the Red Cross, and United Nations agencies such as UNICEF. Since 1954 such donations have totaled $2.1 billion. About 37 million school children are being fed through programs under titles 2 and 3. Title 3 also permits barter agreements whereby food and fiber are exchanged for materials such as tin from Bolivia and chromite from Turkey.

Title 4, which permits credit sales of food for dollars, was added in 1959. The title provides for supply arrangements of up to 10 years and extension of credit for as long as 20 years.

Part of the foreign currencies derived from the sale of surplus foods is used to further U.S. commercial, scientific, and educational efforts. These funds have financed trade promotion and marketing research by the U.S. Department of Agriculture, research programs and translation of technical publications by the Department of Health, Education, and Welfare, and translation of nearly 10,000 books and periodicals by the United States Information Agency. Food for Peace sales also finance educational exchange activities, assistance to 38 American-sponsored schools abroad, and establishment of chairs and workshops in American studies at foreign universities.