second type, derived from glucose, protein, and fat, was prepared in the manner described below from solutions which are commercially available in sterile containers and from a neutral saturated coconut oil which can easily be sterilized with safety. The following represents the basic proportions of the ingredients which are mixed in a 5-gallon, sterile flask: 1 l. of 6 per cent infusion gelatin (Knox P-20), 1 l. of protein hydrolysate 5 per cent amino acid solution, 200 cc. of 50 per cent glucose solution, and 100 grams of pure, refined, edible coconut oil.

After thorough mechanical agitation, the mixture is put through the Logeman homogenizer, an instrument so constructed as to make possible sterilization of its working parts. After this procedure of emulsification and homogenization, the material is collected in sterile vacuum bottle dispensers without any other processing and stored in the refrigerator. Refrigeration converts the emulsion into a solid gel which can be restored to the liquid state by warming the dispensing flask in hot water. Refrigerated samples have maintained complete stability after two months storage. Test samples of the pH of the emulsion averaged 6.5. The droplets were smaller in size than that of the canine erythrocyte and were comparable to that of the chylomicra. The concentrations of fat protein and glucose were approximately 5 per cent each and averaged 800 cal./l. The gelatin acted as a stabilizer, partially as a source of nutrient energy, and served to maintain colloid osmotic pressure.

This emulsion was administered intravenously to 7 dogs and 12 hospital patients. In the latter group at hourly intervals for a period of 8 hours during and after the infusion, temperatures, blood pressure readings, and blood samples were taken. In both groups there occurred no serious untoward reactions as were indicated by the latter studies. Two of the animals received 150 cc. of this emulsion (approximately 7.5 grams of fat) daily for 35 days. The others were sacrificed at varying times for evidence of fatty degenerative changes in the liver, lung, brain, and other organs. Microscopic examination of osmic acid, Sudan III, and hematoxylin eosin preparations failed to show evidence of lipoid granulomatosis. Three patients were maintained exclusively on this emulsion during the postoperative period with satisfactory results. One malnourished patient received with benefit a total of 5,500 cc. of the emulsion (approximately 200 grams of fat) intravenously for a period of 8 days. The emulsion caused no thrombosclerotic changes in the recipient vein and was nonirritating when infiltrated into tissue. Vitamin B and methyl donators were supplied to ensure utilization of fat. Penicillin could be added to the emulsion for intravenous administration.

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IN THE LABORATORY

The Importance of Controlling Cooling Temperatures During Embedding in Paraffin

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Many textbooks of microtechnique (*1*) urge the use of only enough paraffin to cover the specimens and the use of cold water for solidifying paraffin in the process of embedding. Johansen recommends using ice water when embedding during hot weather. Unfortunately, our laboratory has found that this is not always sound advice. The use of cold water for solidifying our particular embedding mass had always resulted in cavitation or numerous low-density areas. Usually the cavities were quite large and were located in the central portion of the block, separated from, or adjacent to, the embedded materials. In an attempt to rectify the situation a series of experiments were carried out. In the light of Johansen's recommendation to use ice water for cooling, it should be stated that the experiments were conducted during the month of August, when temperatures in the room where embedding was done were 90–95° C.

In the first experiment an embedding mass[1] with a boiling point of 54–56° C., heated to 56° C., was poured into paper "boats" 2½ inches long and ¾ inch wide. No specimens were embedded. Some of the "boats" were filled (¾ inch thick), others were poured medium full (½ inch thick), and only a thin layer (¼ inch thick) was poured into others. The paraffin in three such sets was solidified in water at 9° C., 22° C., and 34° C., respectively. In each case the surface of the paraffin was blown upon, and as soon as a supporting crust was formed, the boat was submerged for 12 hours. At the end of the 12-hour period, the boats were dried and the paraffin blocks removed.

Upon cutting the blocks it was found that those medium full and full which had been cooled at 9° C. had large (3 mm. in diameter) continuous or discontinuous cavities throughout the central portion. Both top and bottom surfaces were decidedly concave. The corresponding blocks cooled at 22° C. and 34° C were solid and of desirable texture throughout. The top surface of both sets was concave. The bottom surface of those cooled at 34° C. was either level or slightly convex; that of those

[1] A mixture of 360 grams of Parawax 40 grams of bayberry wax, and 80 grams of stock rubber paraffin (1 pound of Parawax, 10 grams of crude rubber, and ½ gram of asphaltum).

cooled at 22° C. was concave but not as deeply so as in the case of those cooled at 9° C. The shallowly poured block cooled at 9° C. was solid and of good texture throughout. The shallowly poured blocks cooled at 22° C. and 34° C. were somewhat frothy with small cavities (less than 1 mm. in diameter) throughout the central portion from top to bottom and to within 3 or 4 mm. of each side. The bottom of the shallowly poured block cooled at 9° C. was slightly concave, at 22° C. flat, and at 34° C. slightly convex.

Solid pieces of paraffin shaped from comparable locations in blocks cooled at 9° C., 22° C., and 34° C. were sectioned at 5μ and 10μ. All pieces cut equally well and formed excellent ribbons. Pieces of each ribbon were placed on dry slides as well as on slides smeared with Haupt's adhesive and flooded with 3 per cent formalin water. The wet slides were placed on a warm plate, and after the ribbons had flattened and the water had evaporated, both sets of slides were examined microscopically at 125 and 537 magnifications. No consistent differences in texture, crystal size, or any other characteristic could be perceived or measured.

After establishing an optimum cooling temperature range (22–34° C.), large, medium, and small pieces of plant materials were embedded in boats poured full and medium full of paraffin. When these were cooled at various temperatures within the range, the same kinds of results were obtained as for the solid paraffin blocks.

It is improbable that all types of embedding masses have the same optimum cooling range. For this reason it would seem desirable, especially if unsatisfactory results have been obtained, to establish a range for cooling the particular embedding mass used. For practical purposes the complete optimum temperature range need not be determined. It may be found that very low temperatures resulting from icing the water will produce the best results in some cases. They certainly do not give acceptable results in all cases.

In order to determine the effect of disturbing the cooler bottom layer of paraffin soon after pouring, a boat was poured medium full of paraffin at 56° C., and the bulb of a glass thermometer heated to the same temperature was moved in and over the cooler layer of paraffin in a manner resembling rather rough orientation of materials. Care was taken not to introduce air into the paraffin by lifting and lowering the bulb of the thermometer above the surface. Before crystals began forming on the surface, the thermometer was removed, and the boat of paraffin was cooled within the optimum temperature range. The resulting block was solid but had scattered, minute, low-density areas throughout. This experiment was repeated using a "cold" (room temperature) dissecting needle. The resulting paraffin block contained small holes in the disturbed layer at the bottom of the block and numerous, low-density, pin-point spots throughout much of the block.

Our experiments have shown, therefore, that (*1*) for consistently good results, paper embedding boats should be well filled with paraffin to a depth of ½ inch or more; (*2*) an optimum cooling temperature range should be determined for each type of paraffin embedding mass used; (*3*) rapid cooling of some embedding masses does not appreciably improve the quality of the block to be sectioned; (*4*) the use of cold water for cooling may result in cavitation and low-density areas; (*5*) paraffin blocks with flat bottoms may be obtained by choosing the correct cooling temperature; and (*6*) material orientation maneuvers should be held to a minimum and be performed with instruments warmed to the temperature of the embedding mass.

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The Demonstration of Naturally-occurring Streptomycin-resistant Variants in the Human Strain of Tubercle Bacillus H-37RV[1]

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The development of resistance by tubercle bacilli to streptomycin is of considerable practical importance. To help elucidate this problem, an attempt was made to demonstrate the presence of naturally-occurring resistant variants in a stock laboratory strain of H-37RV by examining a large number of organisms. Variants highly resistant to streptomycin have been demonstrated in cultures of *Shigellae* (*5*), strains of *Staphylococcus aureus*, *Staph. albus*, *Proteus vulgaris*, and *Escherichia coli* (*4*), and *Hemophilus influenzae* (*1*).

TABLE 1

	Streptomycin (μg./cc.)				
	0.74	0.37	0.18	0.09	Control
Growth after 3 weeks	0	0	+++	++++	++++
	0	0	+++	++++	++++

TABLE 2

	Tubes with growth			% showing growth		
	3 weeks	4 weeks	Total	3 weeks	4 weeks	Total
Series I, 100 tubes	3	5	8	3	5	8
Series II, 40 tubes	2	0	2	5	0	5

Cultures were made in the liquid medium described by Dubos (*3*), using 10-cc. rather than 5-cc. quantities. A single 4-week-old culture of the human laboratory strain H-37RV growing in Dubos medium was used as the source of the test organisms. The concentration of tubercle bacilli (mg./cc.) was determined by turbidimetric readings in calibrated tubes, using a blue filter (420 mμ) in a Lumetron colorimeter. Streptomycin assays on the supernatant fluid were done, using *Klebsiella pneumoniae* #41 as the test organism according to the method described by Alture-Werber and Loewe (*2*).

Series I: Each of 100 tubes of Dubos medium containing 18.5 μg. of streptomycin/cc. was inoculated with 0.1 mg. of the test culture of H-37RV.

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