bled by the end of 14th day of treatment.

Summary. Administration of Benzmalecene alters serum lipids as follows: 1) decreases serum total and esterified cholesterol concentrations; 2) decreases cholesterol and phospholipid content of α (density >1.063 g/ml) and β (density $>1.019<1.063$ g/ml) lipoproteins; 3) increases serum phospholipid and triglyceride concentrations; 4) increases, both absolutely and relatively, cholesterol and phospholipid content of lipoproteins of density <1.019 g/ml; and, 5) decreases C/P ratio in whole serum and in α and β lipoproteins. The compound possesses extraordinary lipid-altering properties which warrant further investigation. However, the increase in low density lipoprotein lipids may be an undesirable effect since low density lipoproteins may play a role in atherogenesis.

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Effect of Phytic Acid on Zinc Availability.* (25498)

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Considering low zinc requirement of laboratory animals fed casein as source of protein it is surprising that, in recent years, deficiency symptoms have appeared in animals maintained under practical conditions(1). This has probably come about because of more common use of soybean meal in rations inasmuch as several investigators have found zinc in soy protein less available than that in animal proteins(2,3,4). Evidence has been presented that heat treatment increases availability of zinc in soy protein(5,6) although this observation has not been confirmed under our laboratory conditions. Phytin accounts for about 70% of phosphorus in soybean meal, and during extraction of soy protein, phytic acid forms a complex with protein(7). That isolated soy protein has a high affinity for zinc led us to test whether or not a casein-phytic acid complex would decrease availability of zinc to the growing chick.

Methods and material. To prepare casein-phytic acid complex, casein was suspended in distilled water to make a slurry, and phytic acid was added equivalent to 5% of dry weight. After standing overnight the mixture was dried in oven at 60°C. A similar complex was prepared with soy protein† using 4% of phytic acid. Phytic acid analyses were performed by the method of Pringle and Moran(8) except that samples were extracted twice for 18 hours. The original soy protein contained 0.5% of phytic acid phosphorus and the casein-phytic acid complex, 0.8%. According to analysis about one-fourth of phosphorus in commercial phytic acid was inorganic, and total phosphorus contents of proteins were: soy protein 0.9%; casein 0.7%; casein-phytic complex 1.8%. Basal diet, type of chicks and experimental conditions were as previously described(9) except that the battery was plastic coated. Other diets fed were modifications of the soy protein basal. Twenty-six % of soy protein-phytic acid complex, 18% of casein and 5% of gelatin, or 19% of casein-phytic acid complex and 5% of gelatin were substituted for soy protein in the respective diets and glucose hydrate was adjusted accordingly. Other additions were made at the expense of glucose.

Results of 2 trials are summarized in Table I. Rate of growth on casein-gelatin basal diet

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†C-1 Assay Protein, Archer-Daniels-Midland Co., Cincinnati, O.

TABLE I. Availability of Zinc for Chick Growth.

Phytic acid-P in diet, %	Supplements	Wt at 4 wk (g) Trial 1	Trial 2
	A. Casein-gelatin basal*		
.0	None	460 (10)†	447 (10)
.0	Zn, 55 p.p.m.‡	469 (10)	446 (10)
.15	Casein-phytic acid complex	206 (10)	153 (7)
"	*Idem*, Zn, 55 p.p.m.	473 (10)	395 (9)
.29	1.2% Ca phytate§		438 (10)
"	*Idem*, Zn, 55 p.p.m.		476 (9)
	B. Soy-protein basal*		
.12	None	162 (9)	122 (9)
"	Zn, 15 p.p.m.	382 (9)	391 (9)
"	" 55 "	473 (10)	440 (9)
.26	Soy protein-phytic acid complex	97 (6)	94 (2)
"	*Idem*, Zn, 15 p.p.m.	227 (10)	201 (9)

* Zinc content of basal diets was 9 ± 0.5 p.p.m.
† Ten male crossbred chicks were started in each group. No. of survivors is shown in parentheses.
‡ Zinc added as $ZnCO_3$.
§ Nutritional Biochemicals Corp.

was not improved by zinc supplementation, and it may be concluded that approximately 9 p.p.m. of zinc as it occurs in casein-gelatin diet is adequate for growth of the chick. Growth on soy protein basal diet, which by analysis contained the same amount of zinc, was markedly improved by zinc supplementation. In agreement with earlier observations (9) addition of more than 15 p.p.m. of zinc was required to give maximum response on this type of diet.

When the casein-phytic acid complex and gelatin served as source of protein, growth rate was about the same as that on basal soy protein diet. These 2 diets contained approximately the same amounts of phytic acid phosphorus and zinc. Supplementation of casein-phytic acid diet with zinc supported near maximum rate of gain. In contrast to phytic acid in combination with casein, calcium phytate added to casein-gelatin basal diet had little or no effect on growth rate.

Chicks that received artificially prepared soy protein-phytic acid complex grew slowly and exhibited severe symptoms of zinc deficiency. When this diet, which contained 0.26% of phytic acid phosphorus as compared to 0.12% in basal soy diet, was supplemented with 15 p.p.m. of zinc, rate of growth was superior to that on basal diet. Thus 15 p.p.m. of zinc more than overcame the depressing effect of added phytic acid protein complex.

Although these results do not prove that the natural inhibitor in soy protein is phytic acid, they strongly suggest that phytic acid is involved in making zinc unavailable. Commercial soy protein used contains about 0.12% of phytic acid phosphorus, and a similar quantity of phytic acid complexed with casein makes zinc less available. The results suggest that phytic acid must be in combination with protein to make zinc unavailable, because addition of calcium phytate to the diet had little or no effect. It is known that phytic acid forms a complex with soy protein(7) and that proteins complexed with phytic acid are resistant to digestion by proteolytic enzymes such as pepsin(10). It is also possible that failure of calcium phytate to make zinc unavailable is due entirely to its insolubility.

Summary. Zinc in isolated soy protein is less available than that in casein. Zinc in a casein-phytic acid complex, which contains an amount of phytic acid comparable to that found in isolated soy protein, is also less available than that in untreated casein. Addition of calcium phytate to casein-gelatin type diet had little or no effect on zinc availability.

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Factors Influencing Degree of Infectivity of Enterovirus Ribonucleic Acid.* (25499)

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I. *Reversibility of Infectivity of Poliovirus Ribonucleic Acid by Changes in Salt Concentration.* A number of investigators found that free ribonucleic acid (RNA) obtained from several different viruses exhibits only approximately 1/1,000th of the infectivity of intact virus from which it is derived. Fraenkel-Conrat(1) suggested that residual ribonuclease (RNAase) activity in his Tobacco Mosaic Virus RNA preparations accounts for at least some of the apparent instability of RNA, and that RNAase activity is particularly marked in isotonic solutions. Koch *et al.*(2) suggested that the lower infectivity of RNA as compared to whole virus might be due partly to a different and probably less efficient mechanism of entry of RNA into the cell. Our experiments were carried out to gain an understanding of factors which influence degree of infectivity of the active portion of RNA. Such information may be expected to throw some light on the explanation of the differential between infectivity titer of whole virus and that of RNA derived from it. This paper presents evidence that loss of infectivity of poliovirus RNA which occurs on dilution in isotonic salt solutions can be recovered quantitatively by addition of salt (NaCl and KCl).

Materials and methods. Infectivity test system. The cell line used throughout was the Fernandes passage line of human amnion cells (3) grown in Eagle's solution with twice the recommended concentration of amino acids and vitamins(4). RNA was inoculated on 3 day-old cells grown on 60 mm Petri dishes, and the plaque count was made 4 days later. Prior to inoculation with RNA the cells were washed 2 x with isotonic solutions. In all respects, other than use of isotonic wash solutions, the methods were identical to those described previously(5). *Solutions used.* "Minus" Hanks Tris (–HT) consisted of Hanks solution minus calcium, magnesium and phosphate, and buffered at pH 8 with Tris buffer. D_3T = 0.9 M NaCl, 8% sucrose, plus 0.1 M Tris buffer adjusted to pH 8 with HCl. E_1 virus is a preparation of partially purified (100 x concentrated) type 1 poliovirus received from Cutter Labs. It is said to have a molarity of approximately 0.88 with respect to NaCl. Infectious RNA was prepared by modification of phenol extraction method of Gierer and Schramm(6). This method has been described(5). *Alcohol Precipitation.* One part RNA was mixed with 2 parts 95% ethanol and kept at 4°C overnight. Usually no visible precipitation occurred, but since infectivity is regularly quantitatively recovered there seems reason to believe that RNA is precipitated by this process and adheres to the glass. The mixture was then centrifuged in a cold centrifuge at approximately 8,000 rpm for 30 minutes. The supernatant was discarded and glass-distilled water added to the residue in volume equal to that of original RNA preparation.

Results. An RNA preparation which has lost its infectivity in isotonic salt solution can regain its full degree of infectivity when diluted further in hypertonic salt solutions. Table I compares degree of infectivity of the same RNA preparation diluted in solutions of 3 different salt concentrations at constant pH. Two different RNA preparations were exam-

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