wise, the minimal dosage schedules which induce withdrawal phenomena have yet to be established.

Two important clinical implications merit brief mention. First, the qualitative similarity of the meprobamate and the barbiturate abstinence syndromes implies that precautions should be taken to minimize both the development of physical dependence on meprobamate and the severity of the withdrawal syndrome; such precautions as those currently used in the case of barbiturates could be expected to be effective. In particular, following a period of chronic administration of large doses of the drug, medication should never be stopped abruptly. The second implication is that demonstration of physical dependence and an abstinence syndrome following abuse of meprobamate suggests that all members of the new classes of tranquilizing agents should be held suspect until definitely proved to be devoid of such undesirable properties.

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22 January 1957

Enlargement of Avian Eye by Subjecting Chicks to Continuous Incandescent Illumination

During studies on the effect of different durations of diurnal artificial illumination on the growth of chicks, it was observed that the eyes of chicks subjected to continuous light appeared to be flattened. This condition was manifested by reduced depth of the anterior chamber and the apposition of the periphery of the iris to the cornea.

Because of these observations, the eyeballs of chicks from two different lighting treatments were subjected to certain measurements. Eight chicks that were exposed to continuous incandescent illumination and eight chicks that received such light for 12 hours daily provided the material for the study. Each pen, from which all natural light was excluded, received artificial light from a 60-watt, frosted incandescent lamp with a light intensity of 2 to 3 ft-ca measured at the bird height. The chicks were 6 weeks of age when they were sacrificed, and they had been on the two different light treatments since the day after hatching.

The average weight of eyeballs removed from chicks that had received continuous light was about 38 percent greater than the weight of eyeballs of chicks that had received only 12 hours of artificial light each day (Table 1). When the weight of the eyeballs is expressed as a percentage of body weight, there is still a large difference between the two treatments. Thus the data show that an enlargement of the eyeballs of chicks was produced by continuous artificial light.

It was of interest to determine whether the increased size of the eyeballs was brought about largely by an increase in tissue water content or by an increase in both water and tissue dry matter. When fluid was drawn from the posterior chamber of the eye by a hypodermic syringe, about twice as much fluid could be extracted from the eyeballs of chicks that had been subjected to continuous light (0.80 ml per eye) as could be extracted from the eyeballs of chicks that had been subjected to 12 hours of diurnal light (0.45 ml per eye). When the dried weight of the eyeballs is expressed as a percentage of the live body weight, there is only a slight difference between the two treatments. These results show that the increased size of the eyeballs of chicks that received continuous light was caused primarily by an increased accumulation of fluid.

It was found that the average diameter of eyeballs enucleated from chicks that received continuous light was 2.4 mm larger than that of eyeballs from chicks that had not received continuous light. Although average depth was also slightly increased, it appeared that the major change was in the diameter of the eyeballs.

A peculiar enlargement of eyeballs in chicks caused by feeding them a high level of glycine was reported by Groschke *et al.* (*1*). In these studies, the eyes were greatly enlarged by the addition of 8 percent free glycine to a purified diet. When a similar amount of glycine was added in the peptide form, no such enlargement was observed.

The data presented in this report (*2*) indicate that investigations on the effect of continuous illumination on the eye might well be expanded to determine the mechanism of the development of the abnormality. A study of the relationship of this light-induced abnormality to that of eye abnormalities in other species, including man, would be of interest.

Table 1. Effect of length of diurnal incandescent illumination period on eyes of chicks.

Item	Daily illumination period	
	24 hours	12 hours
No. of chicks	8	8
Av. body weight at 6 wk (g)	760	733
Av. weight of eyeballs (g)	4.83	3.50
Av. weight of dried eyeballs (g)	0.515	0.465
Av. diameter of eyeball (mm)	17.6	15.2
Av. depth of eyeball (mm)	12.9	12.1

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Effects of Embryonic Age on Potency in Tissue Culture of Embryonic Nucleoprotein Fractions

In the preparation of various biological growth media, and especially those for tissue culture, it is customary to use whole embryo extract as a source of growth-promoting factors (*1*). The choice of age of chick embryo used for making the extract is commonly one of convenience, usually embryos of 8 to 11 days (*2*). However, Gaillard (*3*) and Fowler (*4*) found that growth of chick heart fibroblasts in plasma culture was stimulated more by saline extracts of 14- to 18-day chick embryos than by similar extracts of 8- to 11-day embryos.

Recently a streptomycin-precipitation procedure was developed using 12-day chick embryos for isolation of the high-molecular-weight growth factors from whole-embryo extract (*5*). It seemed of interest to determine whether this active nucleoprotein fraction (NPF) would show a similar increase in potency or specific activity—that is, biological activity at the same concentration with in-

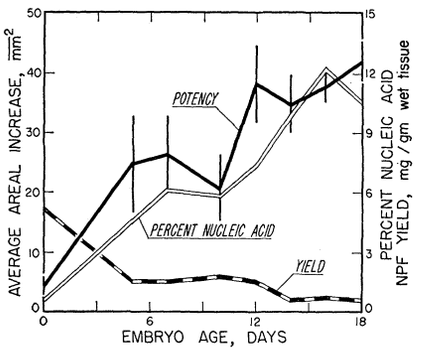

Fig. 1. Changes in yield, potency, and nucleic acid content of the nucleoprotein fraction (NPF) with age of embryonic source tissue.

crease in age of the embryo. It was further hoped that the study might disclose a source of the nucleoprotein fraction with higher yield and greater potency than 12-day embryos (*6*).

Using this streptomycin procedure, nucleoprotein fractions were prepared from nonfertile egg yolk and chick embryos that were 5, 7, 10, 12, 14, 16, and 18 days old. The egg white was devoid of streptomycin-precipitable material of appreciable quantity. The tissues were homogenized in borate with the aid of a Waring Blendor or Teflon-glass homogenizer. Streptomycin was used to precipitate the nucleoproteins from the borate extracts. Suspension of the nucleoprotein fraction in 1*M* NaCl was followed by dialysis against 1*M* NaCl and clarification in the ultracentrifuge. The clear solution was dialyzed against Gey's solution and finally was clarified in the ultracentrifuge. Dry weights and ultraviolet absorption spectra were obtained from aliquots of the clear fractions prior to culturing.

Tissue-culture assays were made using standard techniques (*7*). Fresh explants of 10- to 14-day chick embryo heart were singly cultivated in D-3.5 Carrel flasks at 37°C for a 7-day period. The basal assay medium consisted of a chicken plasma clot in which an explant (1 mm^3) was embedded, with a fluid supernatant of 20 percent horse serum and 80 percent Gey's solution. The nucleoprotein fractions were incorporated in the Gey's solution at the appropriate concentrations. Antibiotics were routinely incorporated into the media. Each test fraction was assayed in a series of six Carrel flasks, and each group of tests included a background control set of six flasks. Supernatant fluids were changed at 4 days, and cultures were traced and terminated at 7 days. Areal increases over and above the control values at 7 days were determined, and statistics (mean value and standard error) were calculated for each series of six cultures.

The average areal increase for the nucleoprotein fractions at 0.4 mg/ml in culture is shown as a function of embryonic age in Fig. 1. The points represent the averages of two extraction series. A general increase of areal outgrowth with increasing embryonic age is to be noted. The differences become significant ($P = 0.02$) when the 10-day nucleoprotein fraction is compared with the 16- or 18-day fraction.

The yields of nucleoprotein fraction per gram of wet starting tissue were calculated and plotted in Fig. 1. The steady decrease in yield with age may be partly accounted for by the difficulties of homogenization and extraction of the older tissues.

Ultraviolet absorption spectra of all the nucleoprotein fractions were similar to the spectrum of the 12-day fraction already published (*5*) with the exception of that of the egg yolk fraction. The latter had a maximum at 280 mμ, indicating a preponderance of proteins and a relatively small quantity of nucleic acids.

The content of total nucleic acids as percentage of dry weight was determined on 5 percent perchloric acid extracts (*8*) by comparing their ultraviolet absorption at 260 mμ with that of Schwartz ribonucleic acid standards. The results are shown in Fig. 1. A steady increase with age of total nucleic acids is to be noted. The nucleic acids are largely ribonucleic acid; previous analyses on the 12-day chick embryo nucleoprotein fraction disclosed that deoxyribonucleic acid accounted for less than one-tenth of the total nucleic acids (*5*).

The results indicate that the high-molecular-weight growth factors are present at all ages and do indeed contribute to the increased potency of whole extracts of older embryos as found by Gaillard (*3*) and Fowler (*4*). However, the decrease in yield of nucleoprotein fraction with age would indicate that the 12-day embryo is the best and most productive source. The parallel increase of nucleic acid and potency of the nucleoprotein fractions may be fortuitous and may reflect only an increase in quantity of nucleic acids in the enlarging embryo since the streptomycin precipitation carries down free nucleic acids as well as nucleoproteins (*9*). Other investigations on adult tissue nucleoproteins (*10*), as well as on embryonic nucleic acids (*5, 11*), indicate that nucleic acids as such are inert in this type of tissue culture. Preliminary experiments indicate that the protein portion of the nucleoprotein fraction is active (*12*). An alternative explanation for the parallel increase of potency and nucleic acid content is possible since unpublished experiments on the stabilization of the nucleoprotein fraction suggest that an increase in nucleic acid content may promote increased protection or stabilization of the active nucleoprotein fraction during isolation procedures. The role of nucleic acids as stabilizers during isolation is being actively investigated.

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Effect of Zymosan on Bacteriophage Clearance

Soon after the discovery of bacteriophage, it became evident that the initial enthusiasm over possible therapeutic application of phage was unwarranted. Among the reasons propounded for the relative lack of success were included the development of resistant bacterial strains, actual deleterious effects due to toxic cleavage products in phage lysates (*1*), failure of phage to reach the focus of infection or to lyse susceptible bacteria *in vivo*, and the inactivation of phage by host defense mechanisms (*2*).

Several investigators (*3*) have reported that human serum, purulent exudates, and other body fluids inactivate phage. The discovery by Pillemer *et al.* (*4*) of the properdin system, a natural defense mechanism effective against a variety of bacteria and animal viruses, suggested to us that this normal serum constituent may be responsible in part for the inactivation of bacterial viruses *in vivo*. Therefore, experiments were initiated to determine whether bacterial viruses are inactivated by the properdin system. While these studies were in progress, Van Vunakis and her associates (*5*) reported the *in vitro* inactivation of *Escherichia coli* phage by the properdin system.

Preliminary experiments in our laboratory revealed that approximately 60-percent inactivation resulted during incubation of staphylococcus phage (typing strain No. 53) with fresh normal rabbit serum at 37°C for 1 hour. Loss