Summary. The various classes of RNA in uninfected and vaccinia virus-infected HeLa cell cultures have been examined through sedimentation analysis in sucrose gradients. Infection with vaccinia virus results in the appearance at 3 hours post-infection of new rapidly labelled RNA fractions. These fractions are distinct from ribosomal RNA and are sensitive to the antibiotic Actinomycin D. The role of these new RNA fractions is discussed.

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The Serum Proteins in the Alcoholic and Mentally Ill Treated with Chlorpromazine.* (29534)

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The interrelationship of biochemical factors with mental and behavioral abnormalities is an incompletely understood area in spite of the very considerable investigation that it has received and a number of suggestive results. Thus, based in part on an electrophoretic procedure developed by Grunbaum *et al*(1), Fessel and Grunbaum(2) found statistically significant variations from normal of certain blood proteins in various groups of chronically psychotic patients confined in a California state hospital. These involved, especially, deviations in the gamma and alpha-2 bands, and suggested among other things, an elevated macroglobulin level.

In continuation of this type of investigation, a broader group of patients involving several types of psychotics and chronic alcoholics was subjected to a similar electrophoretic analysis for the blood protein fractions. In addition, the effect of chlorpromazine on the serum protein distribution of these patients was studied because of the beneficial effects of this drug on the mentally ill. The parameter for normal serum protein values was established by Grunbaum(3) on a population of 221 Caucasian males, the data serving as a basis of comparison in evaluating the proteins in the sera of the alcoholic and the mentally ill.

Experimental. The subjects for this inves-

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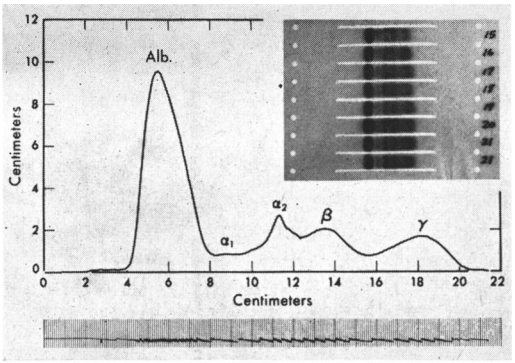

FIG. 1. Electrophoretogram of 8 sera samples (insert) and densitometric tracing with integration of sample No. 20.

tigation were patients (both chronic alcoholic and psychotics) from the Veterans Administration Hospital, Palo Alto, Calif., Menlo Park Division. Serum samples from these patients were collected over a period of 9 months. A total of 109 sera were collected during this period, including 3 control sera, obtained from the V.A. hospital personnel.

Freshly drawn blood from the patients was allowed to clot for one hour, after which it was centrifuged. The serum was separated and deep frozen at once. The clot was discarded. When 6 or more samples accumulated, they were brought to Berkeley under refrigeration and kept in deep freeze until subjected to electrophoresis.

The procedure for electrophoresis was that described by Grunbaum *et al*(1), with the exception that 20 minutes was allowed for electrophoresis instead of 15 minutes. This gave a somewhat better resolution for visual observation.

Veronal buffer of 0.06 M ionic strength at a pH of 8.6 and 250 volts were employed routinely.

According to the design of the apparatus,‡ 8 different sera were run simultaneously. A typical electrophoretogram of 8 people and a densitometric tracing of one of these is shown in Fig. 1.

Results. The results of this study are concerned with the relative distribution of the 5 major fractions found in human serum, namely: albumin, and alpha-1 (α_1), alpha-2 (α_2), beta (β), and gamma (γ) globulins. These values were recorded for each subject. Each subject's condition and whether or not he was on chlorpromazine treatment was also indicated. All the subjects were males from 35 to 50 years old, except for one female control and one 60-year-old subject. In order to make a statistical analysis of variance possible, the subjects were divided into 3 major groups: (1) catatonic schizophrenics, (2) alcoholics, and (3) "others."§ The last is an inclusive group of patients with a variety of mental illnesses. These included patients with paranoia, depression, epileptic psychosis, hebephrenia, character disorder, as well as border line psychotics with an ill defined diagnosis. These three groups represented a sufficiently large population sample for statistical analysis.

A statistical analysis of variance was performed on each group and for each serum protein fraction. In addition, the analyses of variance were supplemented by Scheffe's multiple comparison technique for significant differences at less than the 5% level. The comparisons were among all 3 populations studied with that of a "normal" population, consisting of 221 Caucasian males(3).

It was found that the diagnostic groups (catatonics, alcoholics and "others") differ at the 5% or less significance level from the normal as shown in Table I.

Generally the albumin fraction decreased and the globulins increased wherever significant differences were found.

Perhaps the most interesting finding of this study is the reversal to a normal serum protein distribution of those patients on a continuous medication of chlorpromazine. However, due to the small sample size of subjects, it was not possible to perform a 2-way analysis of variance between diagnosis

‡ Beckman MicroZone Electrophoresis System, Beckman Instruments Inc., Fullerton, Calif.

§ Classification of the chronic mental patients of VAH, Palo Alto, California Menlo Park Division, into 3 diagnostic groups designated as catatonics, "other" psychotics, and chronic alcoholics was done by Fred M. Forrest, M.D., Staff Psychiatrist, prior to electrophoretic investigation of the sera. Diagnoses were made on the basis of the patients' hospital records and clinical symptomatology.

TABLE I. Significant Differences of Serum Protein Fractions Between Diagnostic Groups and a Normal Population.

	Catatonics	Alcoholics	"Other"
No. of patients	14	13	29
albumin	4 (—)	None	4 (—)
α_1 globulin	None	"	4 (+)
α_2 "	"	"	None
β "	"	4 (+)	4 (+)
γ "	2, 3, 4 (+)	None	None

Numbers refer to diagnostic groups, as follows:
1 = catatonics
2 = alcoholics
3 = "other"
4 = normal
(—) = decrease of serum protein fraction
(+) = increase of serum protein fraction

and treatment with chlorpromazine. The degree of reversal to normal on treatment with chlorpromazine was in some cases quite dramatic.

At least 10 subjects on whom it was possible to perform serum analysis over a period of several months and who were continuously on chlorpromazine showed this normalizing effect. Other patients either did not respond in this short period or their serum protein distribution was not significantly different from normal.

One patient, C. R., was the only alcoholic whose serum showed an elevated gamma globulin and low albumin in February 1963. Clinically he was undernourished and underweight. He was given chlorpromazine and his serum was again examined in March, April, May, and August. In the first 2 months, there was no apparent change, whereas in May there was an appreciable reduction in gamma globulin and a corresponding rise in albumin. In August, after about 7 months from the first serum analysis, the serum protein ratio of C. R. was that of a normal person.

The return to normal of the serum proteins coincided with a considerable improvement of the physical health condition of the patients. In all the catatonics, a marked improvement was also noted in their mental condition. Unfortunately, at this date there are not enough data available on other patients which might yield similar observations as that noted on the effect of chlorpromazine. Many of the sera of patients examined twice were taken at too short an interval to observe any effect.

Discussion. In this study, 9 out of a population of 14 catatonics showed a reversal to normal serum protein on treatment with chlorpromazine. Further research will still be required to determine the reasons for this reaction.

The findings of hyper-gamma globulinemia in catatonics is in agreement with the previous report of Fessel and Grunbaum(2). On the basis of their findings, they considered the possibility of a macroglobulin disturbance playing an important role in the pathogenesis of some psychoses. Their conclusion was that more information is required before the serum protein disturbance in chronic psychoses can be satisfactorily explained.

The alcoholics in this study and the psychotics other than catatonics had a significantly elevated serum beta globulin.

This study did not establish electrophoretic patterns which could be used in place of a clinical history to diagnose alcoholism or any kind of mental illness. Normal individuals may differ widely in the sera(4) and whole blood(5) composition. The normal range for any given individual is probably very small. A radical change in this individual's pattern would suggest an abnormality. However, if the new pattern fell within the normal range of a large population, the abnormality would not be detected. It is for this reason that in a given individual, a definitive diagnosis by electrophoretic methods on serum proteins alone is not now possible.

In future studies it may be possible to establish characteristic profiles by adapting specific and sensitive staining techniques that would bring out additional components which have definitive biological activity, such as enzymes, hormones and a variety of protein complexes. As the number of identifiable components is increased, the chances of establishing characteristic profiles for certain diseases will also increase.

In conclusion, the results demonstrate abnormal patterns of protein distribution in the sera of 3 types of mentally disturbed patients. These 3 types do not necessarily rep-

resent well defined disease categories, but rather patterns of abnormal behavior grouped together by clinical designations of practical usefulness, but not of well defined etiological and theoretical significance. It is possible that the differences in serum proteins demonstrated in this study correlate with as yet unknown behavioral and metabolic differences rather than with specific diagnostic categories.

It will be of considerable interest to determine in future studies whether or not significant variations in serum proteins could be demonstrated in those individuals whose behavior disorders are manifested by violence and criminality.

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Oncogenic Activity of Adenovirus 12 in Thymectomized BALB/c and C3H/HeN Mice. (29535)

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Human adenovirus 12 is oncogenic in Syrian hamsters(1,2) rats(3), C3Hf/Gs and C3H/Bi mice(4,5) and *Mastomys*(5). Yabe and his associates(4) also inoculated DBAf and Af mice with adenovirus 12; however, no tumors were produced. The present report describes the results of inoculation of adenovirus 12 in newborn BALB/c, C3H/HeN, dba and AL/N mice. Tumors were produced in the BALB/c and C3H/HeN mice, but this occurred only in animals thymectomized on the day of birth.

Materials and methods. Animals: Pregnant C3H/HeN, BALB/c, AL/N and dba mice were obtained from the Animal Production Unit, Nat. Inst. of Health. *Virus*: Human adenovirus 12 was obtained from American Type Culture Collection. It was passaged and titrated in primary cultures of human embryo kidney (HEK) using methods described previously(5). *Methods of inoculation*: Newborn animals (less than 24 hours old) were inoculated subcutaneously with 0.05 ml of virus preparation. *Thymectomies*: Some litters of newborn BALB/c and C3H/HeN mice were thymectomized within a few hours of birth. With aseptic technique, the skin and sternum of the chest were incised and the thymus removed by forceps and, in some animals, by means of suction. The sternum and skin were then brought together and closed with collodion. Sham thymectomies were performed by incising and then closing the skin and sternums without removal of any tissue. *Necropsies*: Complete necropsies were performed on all animals killed or found dead. Tissues for histopathologic examination were obtained from all viscera and from the brain. They were fixed in formol-sublimate and stained with hematoxylin and eosin.

Results. In the first experiments, 4 litters each of newborn C3H/HeN, BALB/c, dba and AL/N mice were inoculated subcutaneously with $10^{7.15}$ tissue culture infectious doses ($TCID_{50}$) of adenovirus 12. 25 C3H/HeN, 28 BALB/c, 25 dba and 22 AL/N

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