nique in the over-all procedure. The activities are reported as final corrected counting rates per minute in the samples (*6*).

The data from the first series of experiments are given in Table 1. It may be seen that the incorporation of activity into the trichloracetic acid precipitable protein is a slow and continuing process that is dependent on the utilization of oxygen by the tissue. The low values obtained in nitrogen and after very short incubation periods serve as a check on the efficiency of the washing procedure and demonstrate that this uptake is not a simple adsorption on the protein structure. Presumably because of individual differences between tissue slices, the scatter of the data accumulated to date does not allow the shape of the curve of uptake with time to be determined. It could be expected to go through a maximum and fall toward zero, because the net over-all process is proteolysis.

TABLE 1

No. of slices	Activity added (counts/ min.)	Vol. of liquid bathing slices (cc.)	Time	Gas phase	mM CO_2 derived from protein hydrolysate	Total counts/ min. incorporated into protein
2	21,000	1.1	1 min.	Air	0.103	12
2	21,000	1.1	1 "	"	0.121	13
2	21,000	1.1	4 hrs.	Nitrogen	0.124	12
2	21,000	1.1	4 "	"	0.091	4
2	21,000	1.1	4 "	"	0.146	0*
4	42,000	2.2	4 "	"	0.147	0
4	42,000	2.2	4 "	"	0.189	20*
2	21,000	1.1	2 "	Oxygen	0.115	102
2	21,000	1.1	2 "	"	0.131	150
2	21,000	1.1	2 "	"	0.112	126
2	21,000	1.1	4 "	"	0.144	916†
2	21,000	1.1	4 "	"	0.125	160
2	21,000	1.1	4 "	"	0.102	166
2	21,000	1.1	4 "	"	0.146	76*
4	42,000	2.2	4 "	"	0.153	188
4	42,000	2.2	4 "	"	0.146	128*

* The pH of the liquid phase in these experiments was 6.96; that in all other experiments, approximately 7.25.

† This value is thought to be greatly in error. It is included in the interest of completeness.

Because of the variety of reactions in which alanine may participate, detection of activity in protein does not prove that alanine molecules, as such, have entered the peptide chain. Preliminary experiments, in which relatively large quantities of nonradioactive *dl*-alanine were added to a tagged hydrolysate and the alanine crystallized three times, do indicate that a large percentage of the activity in the hydrolysate can be accounted for as alanine. Small amounts of activity were found when similar crystallizations were conducted with *l*(+)-glutamic acid and with *dl*-aspartic acid. Whether this activity represents contamination with alanine cannot yet be stated. It would be expected, in the light of the recent work of Anfinsen, *et al.* (*1*) on the incorporation of activity from bicarbonate ion into the glutamic and aspartic acid fraction of protein, that a similar path to these two species would be available by way of pyruvic acid derived from alanine. No activity was found in glycine, the only other fraction so far investigated.

We regard as slight the possibility that activity has been incorporated by transcarboxylation with the original peptide chain, particularly in view of the reappearance of the activity in the alanine fraction. However, this possibility will be investigated with amino acids tagged elsewhere than in the carboxyl group.

Although the pathway by which alanine is incorporated into the proteins of liver slices remains obscure, it seems clear that under the present experimental conditions little or no incorporation is possible in the absence of oxygen. All experiments completed to date confirm this observation. This finding is compatible with theories of protein synthesis which postulate coupling with energy yielding reactions (cf. Lipmann, *4*).

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Isolation of Avian Pneumoencephalitis (Newcastle Disease) Virus From the Yolk Sac of Four-Day-Old Chicks, Embryos, and Infertile Eggs

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Beach (*1*) has reported the isolation of pneumoencephalitis virus from ovarian tissue of a hen, while Jungherr (*2*) and Van Roekel (*3*) have obtained the virus from fresh eggs. These findings show that the virus may be present in hatching eggs but do not provide evidence that it will survive the incubation period and result in chicks being infected when hatched.

In the experiments reported herein, the four-day-old chicks, the embryos, and the infertile eggs used for virus isolation trials were from parent stock known to be affected with pneumoencephalitis. The chicks were hatched from eggs obtained when egg production was severely depressed as a result of pneumoencephalitis. The embryos, from the same parent stock as the chicks, were from eggs collected when the hens were in the recovery period and the rate of egg production was returning to normal. The infertile eggs, from a second flock of breeders, were collected immediately prior to observed clinical evidence of infection, during the outbreak and through the recovery period.

The virus of pneumoencephalitis was isolated by chick embryo culture from the yolk-sac content of 6 four-day-old chicks and from embryos of the succeeding hatch dead on the 15th day of incubation.

Fifty eggs from the second breeding flock were incubated at this laboratory. The virus was isolated from the pooled con-

tents of 13 eggs which were found to be infertile when candled on the 7th day. Twenty live embryos which were sacrificed and 17 chicks which were allowed to hatch were examined without evidence being found that the virus was present. The chicks remained normal during the 21 days they were held for observation, and their sera were negative when tested for hemagglutination-inhibition antibodies.

It is of interest that clinical evidence of pneumoencephalitis infection was not observed in the flock of four-day-old chicks until several days after the 6 birds from which the virus was isolated had been selected. Two additional lots of chicks from the same hatch delivered to other farms were reported to have remained free from infection.

The isolation of pneumoencephalitis virus from the yolk sac of four-day-old chicks, chick embryos, and infertile eggs further emphasizes the possibility that hatching eggs may be a medium of transmission of the disease from breeding hens to their offspring.

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Antibacterial Action of Inactivated Ergosterol in the Guinea Pig and Man

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The antibacterial action, *in vitro*, of ergosterol and related substances has been reported (*3*). Of these substances, vitamin D (activated ergosterol) has found clinical use in tuberculosis and has often caused toxic symptoms (*1*, *2*, *4*).

In guinea pigs we have been able to show that inactivated ergosterol, like vitamin D (activated ergosterol), suppressed tuberculosis; unlike vitamin D, it never caused any toxic symptoms.

Twenty-two guinea pigs infected with virulent tubercle bacilli were injected with 20 mg. of inactivated ergosterol in oil, or 76 mg. vitamin D (viosterol) in oil, and 11 untreated pigs were used as controls. Following the injection of vitamin D up to several days, a change in the appearance of the guinea pigs receiving this substance was noted: their hair stood up, and the animals shivered, had diarrhea, refused food, and lost weight. No such change occurred after inactivated ergosterol injection. The tubercles present on autopsy were fewer in pigs treated with vitamin D than in the others.

In a second trial, infected guinea pigs received a larger dose of inactivated ergosterol (100 mg.) and less vitamin D (15 mg. D_2). These doses were repeated for three consecutive weeks, with untreated pigs as controls. In those receiving vitamin D_2 the changes outlined above occurred after each injection. No such changes were noted after the much larger dose of inactivated ergosterol. On autopsy there was more suppression of the tuberculosis in the guinea pigs treated with the larger doses of inactivated ergosterol than in those receiving smaller doses of vitamin D.

In man, 300–500 mg. of inactivated ergosterol were injected intramuscularly weekly in cases of pulmonary tuberculosis. Four cases treated thus for 6 months showed more improvement than could be expected by bed rest alone; there was retrogression and absorption of the predominantly exudative and productive disease, with conversion of the sputum to negative for tubercle bacilli (except one case with a cavity over 4 cm. in diameter).

The intravenous injection of inactivated ergosterol did not result in an exacerbation or local reaction, as seen after intravenous vitamin D application.

There were never any ill effects after injection of inactivated ergosterol, regardless of the route of injection or the type of lesion treated.

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Prothrombokinase and the Three Stages of Blood Coagulation[1]

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For four decades a two-stage theory of blood coagulation has held sway. Reviews and textbooks have clung to it for lack of agreement among investigators of the problem concerning further detail. But now sufficient unanimity is emerging to justify reformulation of the basic theory.

When plasma is diluted with distilled water and the pH brought between pH 5.0 and 5.5, the resulting euglobulin precipitate contains the essential core of the blood-clotting system. A solution of this euglobulin preparation clots promptly following the addition of ionic calcium. Even in this somewhat simplified form, the coagulation process can be shown to involve three distinct reactions:

(1) Prothrombokinase	→ Thrombokinase	(in the presence of calcium)
(2) Prothrombin	→ Thrombin	(in the presence of thrombokinase and calcium)
(3) Fibrinogen	→ Fibrin	(in the presence of thrombin)

Evidence that blood plasma contains an inactive form of thrombokinase was available as early as 1916 (*2*); and Bordet long ago detected a preliminary reaction occurring before the development of thrombin (*1*). That these important concepts have gained ground so slowly has been due to difficulties both of interpretation and technique. Among the chief of these has been the lack of a satisfactory procedure for studying the activation of prothrombokinase. Progress toward this objective is included in the following preliminary report.

Bovine prothrombin was prepared by a method involving adsorption on magnesium hydroxide, particular care being

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