liver tissue. It is possible that with higher concentrations of veratrine this might have happened to the action of d-amino acid dehydrogenase on d-alanine. With such concentrations there might have arisen, however, the hazard of incipient precipitation of either protein or drug, a hazard which we strove to avoid.

Quinidine augmented the reaction more strongly than veratrine. Quinine, however, failed to cause any measurable modification of the utilization of oxygen. It must be pointed out, however, that since incipient precipitation of quinine resulted in every experiment the critical concentration may not have been reached. Since the utilization of oxygen in the system containing quinine was not different from that in the control vessels, the material precipitated could hardly have been enzyme-bearing proteins. It was observed repeatedly in experiments employing pH values around 8.0 and a milieu of PO_4 ion that it is not possible to exceed the concentrations of the sulfate of quinine and quinidine which we employed. The stereoisomeric difference between quinine and quinidine is sufficiently fundamental to produce the difference observed.

Conclusions. If one excludes the effects on pH which are produced in the reacting systems by the presence of drugs, the results indicate that ephedrine, morphine, physostigmine, pilocarpine, riboflavin, and sulfanilamide do not modify the catalysis of the dehydrogenation of d-alanine by d-amino acid dehydrogenase. The augmentations produced by quinidine and veratrine, although small, are significant.

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A New Growth Factor for Hemolytic Streptococci.

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Reports have appeared on cultivation of *Str. hemolyticus* in synthetic or semisynthetic media to which growth accessory factors were added. Woolley and Hutchings[1] grew strains of Lancefield's B and D types on a medium consisting of amino-acids, glucose, salts and riboflavin, pantothenic acid, pyridoxin and reduced iron; groups

[1] Woolley, D. W., and Hutchings, Brian, L., *J. Bact.*, 1940, **39**, 287.

A, C and E failed to grow on this medium. McIlvain[2] confirmed these results with the addition of glutamin. Pappenheimer and Hottle[3] grew type A hemolytic streptococcus in a medium which contained, in addition to accessory substances used by Woolley, nicotinic acid, thiamin, biotin and some purines. Woolley[4] reported on the isolation from liver of a new growth factor for *Str. hemolyticus* type A. Type A streptococcus failed to grow in the medium of Pappenheimer and Hottle[3] unless this factor was added.

This report describes the properties of what appears to be a new factor, which gave good growth of *Str. hemolyticus,* type A.

1. The basic medium consisted of:

Acid hydrolized casein	3.0 g
Glucose	1.0 "
NaCl	5.0 "
$Na_2HPO_4 \cdot 12\ H_2O$	2.5 "
KH_2PO_4	0.35 "
$MgCl_2 \cdot 6\ H_2O$	0.3 "
Fe and Mn	trace
H_2O (distilled)	1000 cc

2. The solution (pH 7.4) was sterilized by filtration. The strains were freshly isolated from patients and gave typical β-hemolysis. The strain most used was H2682, kept in broth + 10% serum.

3. The growth factor, derived from tomato juice passed through a meat grinder and filtered. The juice is acid and sterilized by filtering through Seitz filter or triple sterilization in Arnold sterilizer. Addition of 0.05 cc of the neutralized juice to 10 cc of basic medium gives visible growth, while 0.3 cc gives abundant growth. Addition of nicotinic acid, thiamin, riboflavin, ascorbic acid, β-alanin, thioglycolic acid, p-aminobenzoic acid, glutamine, adenine and urazil singly and in combination failed to give visible growth.

The growth substance was purified by the following procedure: (a) Lead acetate in neutral or alkaline reactions precipitates only inactive substances, the active factor remaining in solution. (b) Alcohol (6 vol. to 1) also precipitates the inactive substances. (c) Aceton, 1:1, precipitates only inactive substances; in large excess it precipitates all the active substance. (d) Norit adsorbs the active substance quickly and completely at various reactions (pH 3.0, 7.0, 9.0); Fuller's earth, Kaolin, Kieselgur and talcum fail to adsorb it; animal charcoal adsorbs it only partially and irregularly. (e) Elution from the Norit is affected by the addition of the original volume of

2 McIlvain, H., *Brit. J. Exp. Path.,* 1940, **21**, 25.

3 Pappenheimer, A. M., Jr., and Hottle, G. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 645.

4 Woolley, D. W., *J. Exp. Med.,* 1941, **73**, 487.

80% alcohol containing 1.5% ammonia; about 50% of the active substance can thus be eluted.

These properties were utilized in the purification of the growth factor from the crude tomato juice by the following steps:

1. One l juice concentrated *in vacuo* to 200 cc.
2. One l 96% alcohol added, left over night.
3. The clear decanted supernatant concentrated *in vacuo* to 200 cc.
4. Two l acetone added and left over night.
5. Active precipitate dissolved in 1 l distilled water containing 70 cc H_2SO_4 (pH 2.0).
6. 25 g Fuller's earth added, shaken 30 minutes and filtered.
7. Added 20 g Norit to active acid filtrate, mixture shaken 1 hr and filtered. Filtrate inactive.
8. Norit adsorbate extracted with 1 l alcohol-ammonia sol. during 3-4 hr and filtered.
9. Alkaline filtrate active; immediately concentrated in vacuum.

The neutralized active eluate added to the basic medium without any other growth factor gives visible growth. Addition of riboflavin intensifies the growth, but the addition of the various other growth factors mentioned above had no such effect.

The final eluate had a deep yellow color. The addition of 0.6 cc of this eluate corresponding to 0.3 cc of the original crude tomato extract to 10 cc of the medium yielded good growth.

The purified active substance like that of the crude juice withstood boiling at pH 7.0 or 4.0, for at least 6 hours, but was destroyed in autoclave; at pH 4.0 at room temperature the substance retains its activity for a long time. At pH 9.0 it is much less resistant to boiling; at this reaction the potency is lost after 2 to 3 weeks at room temperature. Boiling half an hour in 0.5 N HCl or 0.5 N NaOH destroys the active substance.

Heating in 3% K MnO_4 or in 3% H_2O_2 destroys the active substance; Br_2 destroys it quickly at room temperature.

The active principle is sensitive to nitrous acid and formaldehyde even at room temperature; 0.1% formaldehyde caused prompt inactivation.

The reactions with nitrous acid and formaldehyde indicate that the active factor contains an NH radical.

The presence of an amino group suggests a relationship to biotin. However, this substance differs from biotin for it is less resistant to heating and particularly to alkaline reactions. The same difference exists in relation to the butyl factor of Woolley[5] and the B-Y factor

[5] Woolley, D. W., McDaniel, L. E., and Peterson, W. H., *J. Biol. Chem.*, 1939, **131**, 381.

of Oxford[6] for anaerobes. It is not identical with glutamine and p-amino benzoic acid because these substances have no activating effect on the growth of *Str. hemolyticus.*

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Comparative Potentiating Effects of Certain Therapeutic Agents on Sodium Evipal Hypnosis.

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Barlow and Gledhill[1] demonstrated the potentiation of isoamyl-ethyl barbituric acid and ethyl (1-methyl butyl) barbiturate on morphine. More recently, Loewe[2] utilized this principle to show that active cannabis preparations, when administered orally to mice, materially prolonged the hypnotic effect of subcutaneously administered pernoston.

We have observed that the intravenous administration of evipal sodium under carefully standardized conditions produces a remarkably consistent reaction on the adult male rat. This is particularly true if the *initial* voluntary movement is taken as the criterion of recovery from hypnosis. Female rats differ materially from males in their reactions to evipal in that hypnosis is more irregular and persists 2 to 5 times as long. Immature animals react inconsistently.

Materials and Methods. Adult male rats, weighing from 325-400 g were used throughout this study; all animals were fasted for 14-18 hours prior to medication. The normal period of hypnosis from a standard dose of evipal sodium administered by the saphenous vein as a 4.0% aqueous solution at an injection rate of 0.1 cc per 15 seconds was determined. This period was estimated within 10 seconds by counting time from the establishment of hypnosis (the completion of the intravenous injection) until the *first* voluntary movement occurred following the injection. A period of 5-7 days was allowed to elapse before the animals were again medicated. The compound to be tested in conjunction with the hypnotic was adminis-

[6] Oxford, A. E., Lampen, J. O., and Peterson, W. H., *Biochem. J.*, 1940, **34**, 1588.

[1] Barlow, O. W., and Gledhill, J. D., *J. Pharm. and Exp. Therap.*, 1933, **49**, 36.

[2] Loewe, S., *J. Am. Pharm. Assn.*, 1940, **29**, 572.