venules; relatively few had migrated into the tissue spaces. This observation suggests that leukocytes migrate more readily through the walls of capillaries whose permeability has been increased. The capacity of cortisone to maintain endothelial impermeability is probably an important factor in its retarding effect upon acute inflammation.

Summary. 1. Various products of protein digestion and extracts of normal tissues caused increased capillary permeability when injected intradermally. This effect was reduced greatly by pretreatment with cortisone. 2. Local edema and leukocytic infiltration developed at the site of the injections. These effects were more acute in the untreated animals. There was evidence that cortisone inhibited the emigration of leukocytes from within the minute vessels.

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Curare-Like Activity of Some Bis-Fluorenyl-Bis-Quaternary Ammonium Compounds. (20966)

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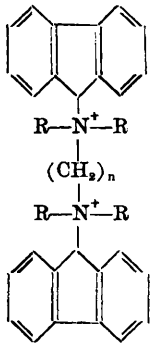
Bovet(1) has proposed a very interesting physical distinction between those agents which induce myoneural junction blockade by either depolarization or stabilization of the end-plate. He proposes the term "leptocurare" for agents which induce blockade by depolarization and for which the molecular configuration usually is long and thin, *i.e.,* decamethonium. "Pachycurare" is proposed for those agents which induce blockade by stabilization of the membrane, such as d-tubocurarine, and for which the molecular configuration is quite bulky in width. In the reports of Barlow and Ing(2,3), Paton and Zaimis(4,5) and in the excellent reviews of Paton(6) and Riker(7), it has been observed that maximum curariform activity, with bisquaternaries, is obtained when the distance between quaternary nitrogen atoms is approximately 15 Å, irrespective of the type of blockade instituted.

This is a report on the neuromuscular blocking properties of a new series of compounds prepared by Gray *et al.*(8), in which the nitrogen atoms are joined by a polymethylene chain and in which one of the groups on each nitrogen atom is a fluorenyl group. The compounds are of interest in that the large terminal groups modify the distance between nitrogen atoms which have been reported necessary for optimal activity. A similar modification of activity by terminal groupings was reported by Wien *et al.*(9) for the ganglionic blocking agents in the methonium series.

Methods. 1) *Mice.* The inclined screen procedure of Thompson(10), as modified by Hoppe(11), was used for the quantitative determination of the potency of these compounds. Concentrations were varied by equal increments so that at least 3 reactive groups were obtained between the ED_0 and the



where R = CH_3, n = 6 (IN-117)
R = C_2H_5, n = 6 (IN-137)
R = C_2H_5, n = 10 (IN-138)

ED_{100}. The mice were run in groups of 10 and the concentration adjusted so that 0.01 ml of solution was administered per 2 g of body weight. The subcutaneous absorption of these compounds was found to be very slow, requiring from one-half to 3 hours for the onset of paralysis; therefore, those mice which became paralyzed and slid off the screen within 3 hours were considered positive reactors. Twenty minutes was sufficient time to obtain maximum reactors by the intravenous route of administration. The ED_{50} and LD_{50} were calculated by the method of Behrens (12). 2) *Rabbits.* Intravenous doses of increasing equal increments were administered to rabbits in groups of 5. Concentrations of solutions were adjusted so that 0.1 cc was administered per kg of body weight. A reactor was considered positive when a "head drop" was obtained to the extent that the head rested on the table top and could not be raised, even by prodding. The dose producing "head drop" in 50% of the animals (HD_{50}) was calculated by the method of Behrens(12). 3) *Dog.* Myoneural junction transmission. Dogs were anesthetized with pentobarbital sodium (35 mg/kg) intravenously. The tendon of Achilles was sectioned just proximal to the calcaneous by cautery, to avoid bleeding. A heavy thread was tied around the tendon, which was then led over a pulley and to which was attached a 200 or 300 g weight. A second thread was tied to the thread of the tendon in such a manner that through a system of pulleys a lever reflected upwards for every contraction of the gastrocnemius muscle. The peripheral end of the sectioned tibial nerve was stimulated electrically with currents just above threshold values at a rate of 15/min. A half-hour period of stimulation was allowed for stabilization at which time the current intensity was redetermined to be certain that it was still close to the threshold value. If the contractions were of uniform height during another 15-minute stabilization period, the determination of the myoneural junction blocking dose of these compounds was begun. Injections were made in the femoral vein at intervals of 3 hours. More rapid injections led at times to a lessening of the dose which would completely block the gastrocnemius twitch. At least 10 determinations were made of the dose which was just below and just above the dose which gave a complete inhibition of the twitch. By this process of "closing-in", reproducibility within 5% was obtained.

Results. Mice. The group of bis-fluorene compounds tested in mice demonstrated activity less than that which was obtained by d-tubocurarine, whether administration was by the intravenous or subcutaneous route. The intravenous ED_{50} values (determined by the inclined screen procedure) of IN-117, IN-137, and IN-138 were 4.3%, 7.5% and 7.7%, respectively, as active as d-tubocurarine. The ratios ED_{50}/LD_{50} of these agents in the above order were 0.72, 0.62 and 0.93. From these ratios it can be seen that there was a very slight spread between the ED_{50} and LD_{50} of IN-138, but an appreciable spread was obtained with IN-117 and IN-137.

TABLE I. Comparative Pharmacology of IN-117, IN-137, IN-138 and d-Tubocurarine.

			d-Tubo	IN-117	IN-137	IN-138
ED_{50}	Mice	I.V.	.054*	1.26	.72	.70
LD_{50}	"	"	.14	1.76	1.16	.75
LD_{100}	"	"	.21	2.02	1.6	
ED_{50}	"	S.C.	.275	238	14.7	
LD_{50}	"	"	.525	240	19.95	
LD_{100}	"	"	.70	400	40.0	
ED_{50}	"	Oral		280		
LD_{50}	"	"		280		
LD_{100}	"	"		400		
HD_{50}	Rabbit	I.V.	.161	0.08	.09	.205
LD_{50}	"	"	.223	0.11	.11	.220
LD_{100}	"	"		0.12	.12	.25
Myoneural junct. block (dog)			.24	0.21	.21	.66

* Doses in mg/kg.

The subcutaneous ED_{50} and LD_{50} of IN-117 and IN-137 differed markedly between the 2 compounds. The ED_{50} of IN-117 was found to be 149 and the LD_{50} 137 times the dose required to obtain these responses by the intravenous routes. For IN-137, the ED_{50} S.C./ED_{50} I.V. and LD_{50} S.C./LD_{50} I.V. were found to be 20 and 17, respectively. It is probable that a slow rate of absorption can account for the very high values obtained with IN-117. That IN-117 has such a markedly slow rate of absorption is interesting since it differs from IN-137 only by the substitution of methyl for ethyl groups on the nitrogen atoms. Since this factor of absorption can occur, extreme caution should be practiced in the quantitative evaluation of "structure-activity" data which are obtained by routes of administration other than the intravenous.

Rabbits. IN-138 responses were similar to those of d-tubocurarine in the rabbit. Doses 3 times greater than the mouse I.V. ED_{50} were required to produce the HD_{50}. IN-117 and IN-137, however, produced the HD_{50} with doses which were 0.06 and 0.12 times their respective I.V. mouse ED_{50}. Onset of action was apparent within one minute after full curarizing doses were administered. In subcurarizing doses the onset was delayed from 2 to 5 minutes. Recovery was noted within 10 minutes in rabbits which lived after a "head drop". The LD_{50} values obtained paralleled quite closely those values obtained for the HD_{50}.

Dog. IN-117, IN-137 and d-tubocurarine produced blockade of the myoneural junction in almost equivalent doses. However, IN-138, the decamethylene analog, could only induce complete blockade, with doses 3 times those of the former agents. Complete recovery of the myoneural junction blockade in dogs after the administration of "effective" doses of IN-117 was between 15-30 minutes. It was found that C-10 (0.2 mg/kg) immediately antagonized the complete myoneural junction blockade induced by IN-117. Neostigmine (0.05 mg/kg) antagonized the blockade for about one minute; the blockade then being again reinstituted. Further administration of neostigmine had no further effect on the blockade.

Other actions. IN-117 was infused into an anesthetized artificially respired dog at various rates over a 6-hour period. After a total of 91.2 mg/kg had been administered, it was found that the H.R. had decreased from 162 b.p.m. to 144 b.p.m. The blood pressure had decreased from 115 mm Hg to 80 mm Hg.

A large dose of epinephrine (0.05 cc of a 1:1000 solution) was administered at the completion of the experiment to determine cardiac irritability. The heart did not go into fibrillation. IN-117 did not cause inhibition of the response of stimulation of the peripheral end of the vagus as reported for curare by Claude Bernard. In atropinized, anesthetized dogs, it was found that the nicotinic blood pressure response of Ach was potentiated by IN-117, the degree of potentiation being greater with increasing doses of IN-117. In mice, the "essential elimination" rate is 0.32 mg/kg/min.

Discussion. A new group of myoneural junction blocking agents is reported which demonstrates myoneural junction blockade in the dog and rabbit when the distance between nitrogen centers is approximately 9 Å. IN-137, in which the nitrogen atoms are separated by a 6-carbon chain and in which the nitrogen atoms contain 2 ethyl as well as a fluorenyl substituent demonstrated the greatest activity in all the animals tested. When the ethyl groups of the quaternized nitrogen are replaced by methyl groups (IN-117) the activity is markedly diminished in the mouse but is retained in the dog and rabbit. The replacement with the methyl groups of IN-117 also demonstrates what very probably is a very marked slowing of the rate of subcutaneous absorption. Doses in the order of 150 times the effective intravenous dose are required to produce 50% responses in mice, whereas with the ethyl analog a dose increment of approximately 20 times the effective intravenous dose is required to produce the same response.

Increasing the chain length of IN-137 from 6 carbons to 10 carbons (IN-138) induces no appreciable change in activity as measured in mice. However, activity is reduced approximately 66% from that obtained with IN-137 when rabbit head drop and dog myoneural

junction blocking doses are compared.

Conclusions. 1. The curariform activity of a new series of bis-quaternary ammonium compounds is reported. 2. Moderate increase of activity is obtained by shortening the distance between nitrogen atoms from approximately 15 Å to 9 Å. 3. Replacement of N-ethyl groups with N-methyl in the 6-carbon chain analog decreases both the activity and the subcutaneous absorption in mice, but has no effect on the activity as measured in dogs and rabbits.

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Peptidases in Isolated Blastomeres of *Mytilus edulis.** (20967)

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Investigations of mosaic eggs of annelids and mollusks in attempts to characterize the various cytoplasmic regions in chemical terms have demonstrated regional differences in pH, rH, enzymes and oxidative metabolism, Spek(1), Ries(2), Berg and Kutsky(3). The following investigation was undertaken to see if regional differences of peptidases might occur in a mosaic egg.

Peptidases have been found in eggs and embryos of invertebrate animals, Holter(4), Linderstrøm-Lang and Holter(5), Holter, *et al.*(6). No correlation of enzymatic activity with cell determination has been shown in the regulative egg of the sea urchin, Holter and Lindahl(7). Alanylglycine peptidase has been found in the mosaic eggs of *Tubifex,* Holter *et al.*(6), and of *Chaetopterus,* Holter(4); however, the localization of the enzyme and correlation with morphological differentiation was not investigated. The eggs of *Mytilus edulis,* used in this study, undergo a mosaic development as shown by differentiation of isolated blastomeres, Rattenbury and Berg (8). The most obvious segregation of factors important to subsequent differentiation occurs at the first cleavage, and methods have now been developed for removal of the egg membranes and separation of the first cleavage blastomeres, Berg(9). Measurements of peptidases in first cleavage blastomeres, reported here, are in part the results of an investigation of the physiological and chemical properties of the isolated early cleavage blastomeres.

Methods. The gametes of *M. edulis* were obtained by allowing individual animals to spawn in dishes of sea water. Vitelline membranes were removed with a membrane lysin and the first cleavage blastomeres separated in calcium free sea water. Peptidases were determined by a modification of the titrimetric method of Linderstrøm-Lang and Holter(10). The segregated blastomeres were cytolyzed in 7.4 μl glycerol-phosphate solution. 7.4 μl of substrate (alanylglycine or leucylglycine) were added and the mixture incubated

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