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1,3-ACETONE BIS (4-HYDROXYIMINOFORMYL PYRIDINIUM) DIBROMIDE (MBM-3): CHEMICAL PROPERTIES, CHOLINESTERASE REACTIVATION, PROTECTIVE ACTION IN EXPERIMENTAL PARAOXON POISONING

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1,3 acetone bis (4-hydroxyiminoformyl pyridinium) dibromide (MBM-3) mp. 218–20° was synthetised from pyridine-4 aldoxime and 1,3 dibromacetone. The UV spectrum of MBM-3 dissolved in n/10 HCl has a maximum at 2820 Å, and dissolved in n/10 NaOH at 3400 Å. The IR spectrum indicates a syn-syn configuration.

The LD_{50} of MBM-3 in white mice is 85 mg/kg (i. v.) and 155 mg/kg (i. p.). At 10^{-5} M concentration MBM-3 did not inhibit the erythrocyte cholinesterase itself but reactivated by 87% the erythrocyte cholinesterase inhibited with paraoxon.

Applied together with atropine it offered in mice a complete protection against 80 LD_{50} of paraoxon.

Trimedoxime (TMB-4) and Toxogonin (LüH 6) belong to the most powerful reactivators of cholinesterases (ChE) inhibited by organophosphorus compounds (1) Both oximes are better antidotes in organophosphorus anticholinesterase poisoning than pralidoxime (PAM-2), but they are also more toxic (2).

In our earlier investigations of 1-phenacyl and 1-benzoylpyridinium and pyridinium-4-aldoxime chlorides we were able to demonstrate that the presence of the C = 0 group diminishes the toxicity in this series of compounds (3). It seemed, therefore, reasonable to expect that replacing one CH_2 group in the trimethylene chain of TMB-4 by a C = 0 group would reduce the toxicity of this oxime.

The present paper describes the synthesis, chemical properties and cholinesterase reactivating action of such compound 1,3-acetone bis (4-hydroxyminoformyl pyridinium) dibromide (MBM-3). This com-

ponud under the designation S-101 has been mentioned by *Hauschild* et al. (4) in a study of a series of newly synthetised oximes, but no data were given about its chemical properties, its toxicity and its reactivating power.

Bearing of mind the possibility of keto-enol tautomerism, there is some similarity between MBM-3 and compounds which carry an OH group in the polymethylene chain. Such compounds were recently synthetised by *Engelhardt* (5).

MATERIALS AND METHODS

Method of preparation

In a three necked flask, equipped with a mechanical stirrer, dropping funnel and condenser, 12,21 g (0.1 mole) of pyridine-4-aldoxime was dissolved in 150 ml of absolute ethanol by vigorous stirring and heating. When all the pyridine-4-aldoxime was dissolved, the mixture was heated to 70° and, during 1 hr, 10.79 g (0.05 mole) of 1,3 acetone dibromide was added. The reaction was allowed to continue for another 5–6 hours within the same temperature range, under vigorous stirring.

After the reaction was completed, the precipitated orange brown powder was filtered off, washed several times with acetone and air dried. Yield 20 g (85% theor.). By recrystallisation from boiling ethanol, with addition of charcoal, orange-brown crystals m. p. 218–220° were obtained (lit. 217–19) (4) the product is soluble in water (18%) and sparingly soluble in ethanol, acetone and ether.

Anal.: $C_{15}H_{16}N_4O_3Br_2$ (460) requires N 11.8% Br 34.8%
found N 12.1% Br 35.6%

Neutralisation equivalent theor. 460.04
found 465.60

The UV spectrum of substance was recorded with Beckmann DU spectrophotometer MBM-3 dissolved in n/10 HCl has a maximum at 2820 Å (log E = 4.44). In n/10 NaOH the maximum is shifted to 3400 Å (log E = 5.13).

The IR spectrum of the substance was recorded with »Unicam SP-200« IR spectrophotometer, in KBr. (fig. 1).

The substance is characterised by intense absorption bands at 3050 cm^{-1} and 1020 cm^{-1}, corresponding to = NOH and N-O stretch frequences: another characteristic band at 1740 cm^{-1}, corresponds to C=O stretch. There are bands at 1080 cm^{-1}, and 840 cm^{-1}, which are not shifted in Nuyol mull, indicating a syn-syn configuration of the oxime.

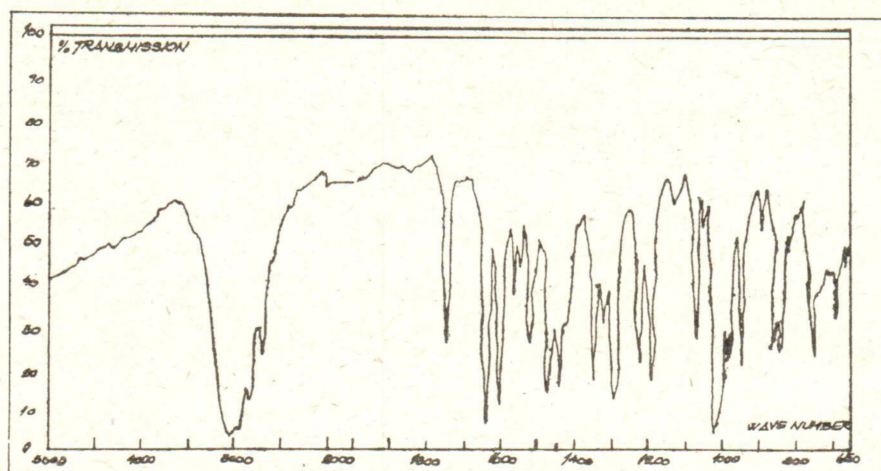


Fig. 1.

Toxicity

The LD_{55} was determined in white mice of either sex, weighing about 20 g. The oxime solutions in water were given intraperitoneally or intravenously (0.2 ml/20 g). The mortality ratio was recorded 24 hours after the oxime injection. LD_{50} values with 95% solution consisting of calculated according to *Litchfield* and *Wilcoxon* (6).

Cholinesterase activity was determined manometrically in a Warburg apparatus at 37 °C, in a freshly prepared buffer solution consisting of 0.164 M NaCl, 0.15 M Na HCO_3 (pH = 7.7) in a nitrogen-carbondioxide atmosphere (95% N_2 + 5% CO_2). The method of determination was described earlier (3). As a source of enzyme we used human erythrocytes. Acetylcholine chloride, in final concentration of 0.01 M was used as substrate.

The protective effect of oximes was tested in white mice (weighing 20 g) poisoned by paraoxon. The 1% stock solution of paraoxon in ethanol was diluted to desired concentrations with isotonic saline and administered subcutaneously. The oxime solutions, together with atropine sulphate (10 mg/kg), or without atropine, were administered intraperitoneally, after the paraoxon injection. The mortality ratio was recorded 24 hours after the paraoxon injection.

RESULTS

Toxicity

LD_{50} values in mice for TMB-4 (m. p. 240–242° C) and MBM-3 after intravenous and intraperitoneal administration are given in Table 1.

Table 1.

Acute toxicity of MBM-3 after intravenous or intraperitoneal administration in mice

Oxime		№ of animals	LD_{50} and its 95% confidence limits (mg/kg)
MBM-3			
	i. v.	30	85 (77–95)
	i. p.	30	155 (124–193)
TMB-4			
	i. v.	30	80 (72–88)
	i. p.	30	145 (120–174)

It can be seen that the toxicity of both compounds is quite the same in this species. The signs of poisoning, in which depressed respiration predominates, are the same with both drugs.

The length of survival after the administration of LD_{50} of oxime was the same for both compounds: about 10–15 minutes.

Reactivation of ChE

MBM-3 and TMB-4 have but little effect on the activity of human erythrocyte cholinesterase in amount to be expected in vivo (10^{-5} M or less) after administration of relatively large nontoxic doses. Higher concentrations (10^{-3} and 10^{-2} M) cause a high degree of enzyme inhibition. MBM-3 is a more potent inhibitor of cholinesterase than TMB-4.

At 10^{-5} concentration which, as mentioned, has no effect on ChE activity, MBM-3 is a potent reactivator of cholinesterase inhibited by paraoxon, in vitro, although not comparable in potency to TMB-4. This is shown in Table 2.

Protective effect

By intraperitoneal administration of 30 mg/kg MBM-3 it is possible to raise the LD_{50} of paraoxon (0,7 mg/kg) about 8 times. Despite this relatively strong protective effect of MBM-3 (without atropine) the

Table 2.

*Effect of MBM-3 and TMB-4 on the activity of erythrocyte cholinesterase inhibited by paraoxon (2.4×10^{-5} M)**

Treatment	Concentration (M)	№ of experiments	Cholinesterase activity (μl CO_2/ml/min)	% reactivation
Nil		12	170.47	—
Paraoxon		9	13 89	—
Par. + MBM-3	10^{-6}	4	94.43	51
Par. + MBM-3	10^{-5}	3	150.23	87
Par. + TMB-4	10^{-6}	4	172.40	101

* Time of reactivation 30′

protective index is much lower than that of TMB-4, which in a dose of 25 mg/kg, can step up the LD_{50} of paraoxon about 30 fold (Table 3).

Table 3.

Influence of MBM-3 and TMB-4 (without atropine) on mortality in mice poisoned with paraoxon

Paraoxon (s. c.)		Mortality *	
mg/kg	multiple of LD_{50}	MBM-3 (i. p.) (30 mg/kg)	TMB-4 (i. p.) (25 mg/kg)
1.4	2	0/6	—
2.8	4	1/6	—
5.6	8	3/6	—
7.0	10	6/6	1/6
16.8	24	—	0/6
21.0	30	—	3/6

* Number of animals dead/number of animals treated. Oximes were administered immediately after paraoxon.

The protective effect of MBM-3 in mice poisoned with paraoxon can be improved considerably if the oxime is given together with atropine. Such a combination of antidotes increases the LD_{50} of paraoxon about 100 times. No mortality in mice was obserwed within 24 hours following 56.0 mg/kg of paraoxon, which means a complete protection against 80 LD_{50} of paraoxon (Table 4).

A combination of atropine with TMB-4 affords about the same degree of complete protection. A number of poisoned animals treated with atropine + TMB-4 remains alive after greater doses of paraoxon than after MBM-3 + atropine combination.

Table 4.

Influence of MBM-3 and TMB-4, respectively, in combination with atropine (10 mg/kg) i. p. on the mortality of mice poisoned with paraoxon

Paraoxon (s. c.)		Mortality *	
mg/kg	multiple of LD_{50}	MBM-3 (i. p.) (30 mg/kg)	TMB-4 (i. p.) (25 mg/kg)
28	40	0/6	—
56	80	0/6	0/6
70	100	5/12	1/12
84	120	6/6	4/6
100	143	—	6/6

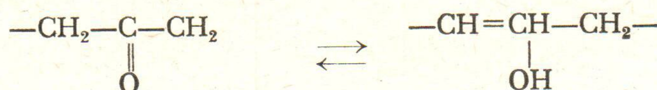
* Number of animals dead/number of animals treated. Oximes together with atropine were administered imediately after paraoxon.

DISCUSSION

The experiments described in this paper have shown that the introduction of a C = O group in place of the central CH_2 group of the trimethylene bridge of TMB-4 has none of the beneficial effect upon the toxicity, that has been observed in the series of phenacyloximes (3). But this change in the molecule of TMB-4 significantly diminishes its protective power in paraoxon poisoning. When given together with atropine, however MBM-3 affords practically the same degree of absolute protection as the combination TMB-4 + atropine. We could not establish that the substitution of the central CH_2 group, in the trimethylene bridge of TMB-4, by the C = O group causes a greater reactivating power which, however, was the effect observed by *Engelhardt* and *Erdmann* (7) upon replacing the same CH_2 group by an oxygen atom. According to these authors the ether —0— atom in the ethylene oxide bridge of LüH 6 has a powerfull inductive effect upon the pyridine ring, pushing the electrons towards the ring, thus lowering the pKa and increasing the percentage of ionized oximate at physiological pH values, which cannot be case in the compound MBM-3.

Engelhardt and *Erdmann* (7) also expresse the opinion that the free rotation of the C-O-C link is greater than of the link C-C-C and this is also a cause for the better reactivating power of LüH 6 over that of TMB-4. But surely, the link C-C(O)-C in MBM-3 is more rigid than either C-C-C or C-O-C and yet the reactivating power of MBM-3 in a series of different phosphorylated ChE is quite equal to that of TMB-4 or LüH 6 (8).

From chemical point of view, introducing the C = O group into the polymethylene bridge offers great possibilities to study the keto-enol tautomerism in this kind of compounds



The I. R. spectrum of MBM-3 in the solid state gives evidence only of the existence of the keto form. We intend to find out in further investigations in which form MBM-3 reacts in solution and *in vivo*.

Moreover MBM-3 offer a very interesting possibility to form intramolecular and intermolecular bonds which according to *Engelhardt* and *Erdmann* (7) are of great importance for the oxime activity.

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Summary

1,3 - ACETON BIS (4-HIDROKSIIMINOFORMYLPIRIDINIJUM) DIBROMID (MBM-3) : KEMIJSKA SVOJSTVA, DJELOVANJE KAO REAKTIVATORA KOLINESTERAZE I ZAŠTITNOG SREDSTVA U EKSPERIMENTALNOM TROVANJU PARAOKSONOM

1,3 aceton bis (4-hidroksiiminoformilpiridinijum) dibromid (MBM-3) tt. 218–20° sintetiziran je iz piridin-4 aldoksima i 1,3 dibrom acetona. UV spektar otopine MBM-3 u n/10 HCl ima maksimum na 2820 Å, a u n/10 NaOH na 3400 Å. IR spektar ukazuje na sin-sin konfiguraciju. LD_{50} u bijelih miševa iznosi 85 mg/kg intravenozno, a 155 mg intraperitonealno. U koncentraciji 10^{-5} M ne dovodi do inhibicije kolinesteraze eritrocita, ali reaktivira kolinesterazu eritrocita inhibiranu paraoksonom za 87%. Dat preventivno miševima zajedno sa atropinom pruža apsolutnu zaštitu od 80 LD_{50} paraoksona.

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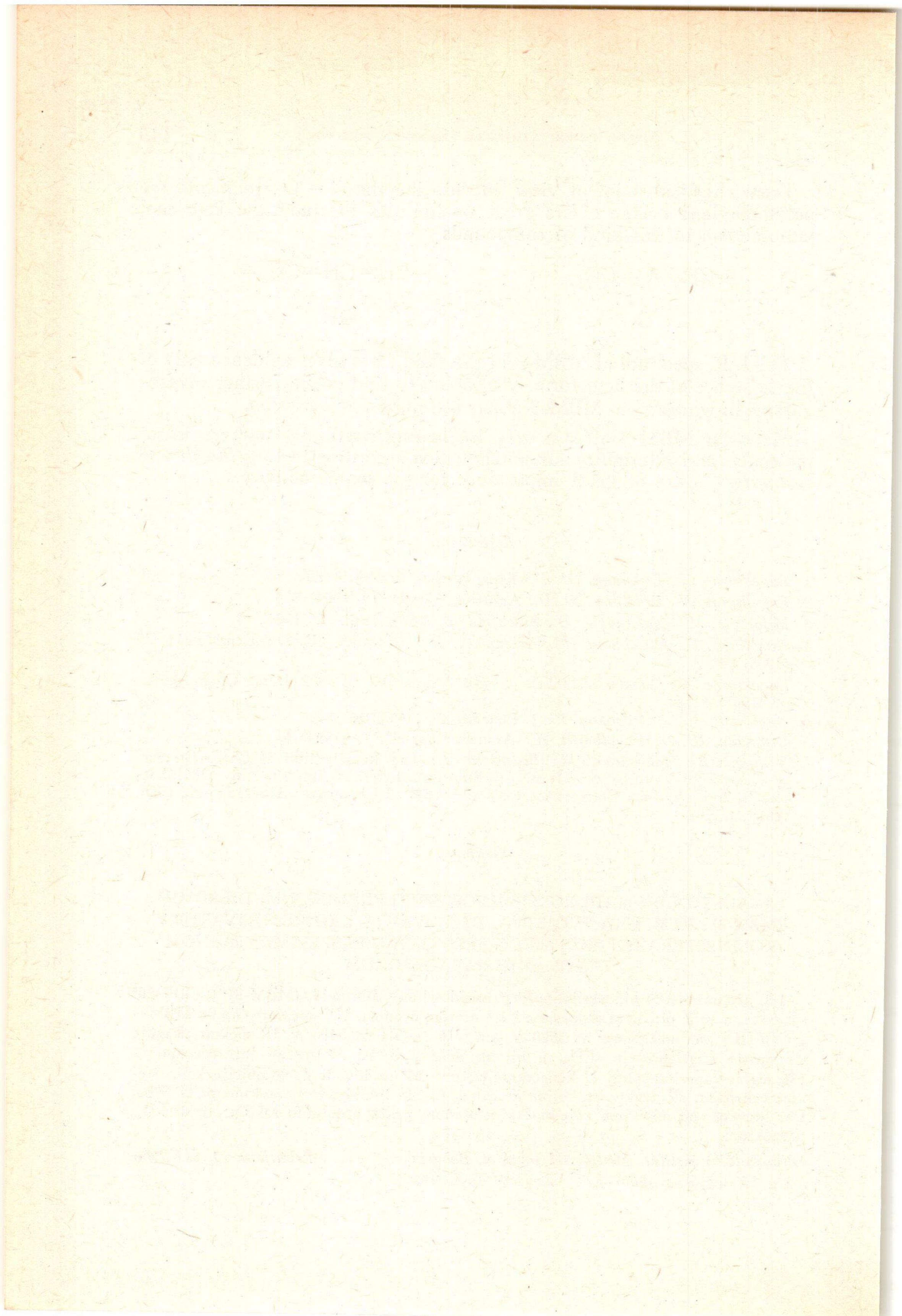
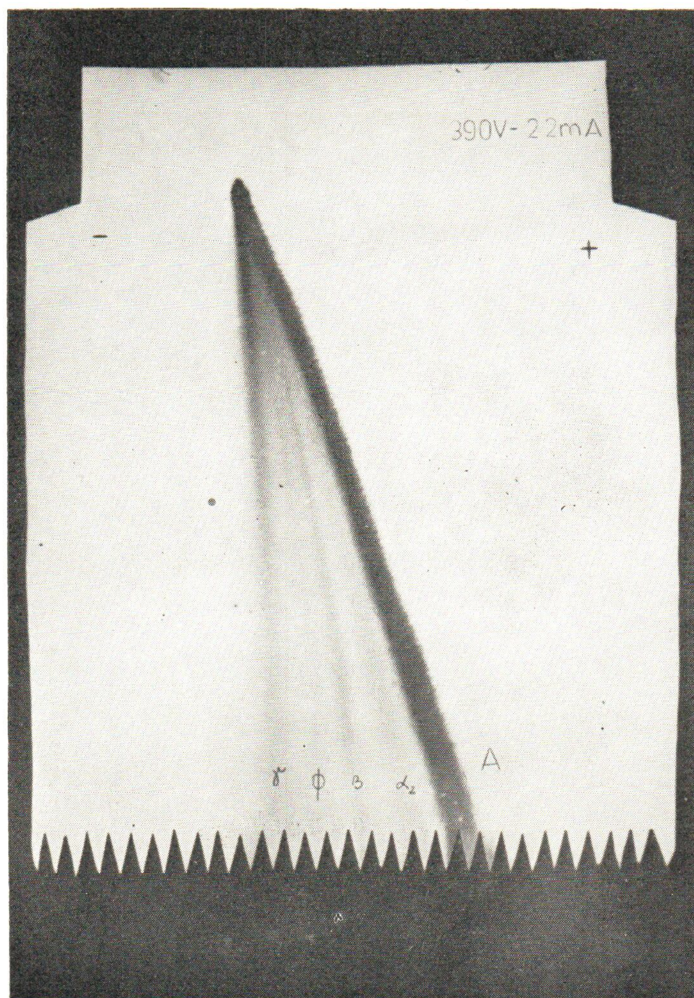
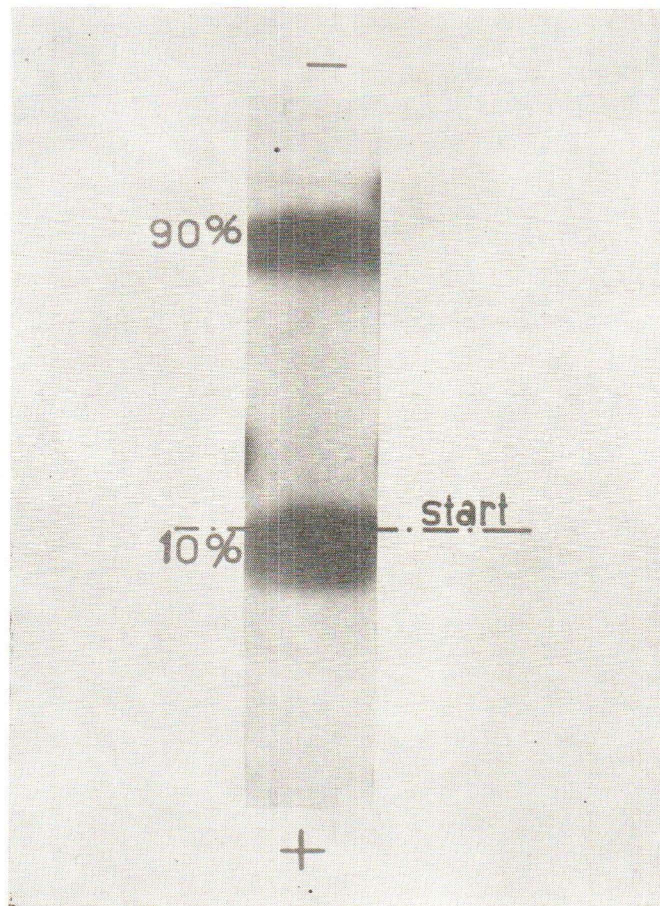




Sl. 2. *Kontinuirana elektroforetska separacija proteina plazme. Pufer: barbiturat pH* 8,6; $\mu = 0{,}025 + 7\ mg^{0}/_{0}\ Ca$



Sl. 3. *Autoradiogram diskontinuirane elektroforetske separacije plazme inkubirane sa Ca-47. Postoci se odnose na Ca-47. Pufer: barbiturat pH* 8,6; μ 0,05 + 7 *mg%* *Ca*