# ON THE ABSORPTION OF SALT SOLUTIONS FROM THE PLEURAL CAVITIES. BY J. B. LEATHES, M.B. (*Oxon.*), F.R.C.S., AND ERNEST H. STARLING.

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IN a paper communicated to Vol. XVI. of the *Journal of Physiology*, "On Absorption from and Secretion into the Pleural Cavity," it was shown that coloured fluids were absorbed by the blood vessels very much more rapidly than by the lymphatics. How far this process was physiological, how far purely physical, how far, that is, the absorption was due to forces developed by the activity of the endothelial cells lining the serous membrane, or forming the walls of the capillaries in the membrane, and how far it could be explained by simple osmosis, was not determined. There was however some evidence to show that, with certain solutions, an active absorption may take place. To settle the question, we have undertaken a series of experiments—the subject of this paper.

A precisely similar question, with regard to the part played by the epithelium of the intestine in the absorption of salt solutions from the bowel, had in the interval been discussed in a paper published in *Pflüger's Archiv* (Vol. LVI.), by Prof. Heidenhain, and this already classical work furnished us with a model for our research. This author introduced salt solutions of known strength, which was different in different experiments, into a loop of intestine shut off from the rest of the intestinal canal by ligatures. After a certain time the loop was opened again and the fluid collected and analysed. Experimenting upon a number of dogs in this way, he found that, while the effects of osmosis could always be recognized and identified, there were certain results which could not be referred to osmosis alone, but were evidence of the existence of some force modifying the action of osmosis. Thus, in the first place, fluids having the same freezing point and therefore the same osmotic pressure as the serum of the animal experimented upon (such fluids, for instance, as 1 % sodium chloride solution, or serum obtained from another dog), rapidly diminished in volume. Again, fluids with a considerably higher osmotic pressure than the serum also diminished in volume; this was the case even with 2 % sodium chloride solution, the osmotic pressure of which is about double that of the serum. And lastly he found that, when he used a solution containing less than 1 % of sodium chloride, and therefore of lower osmotic pressure than the serum, some of the salt was absorbed.

The force which causes absorption under these three conditions is clearly something more than osmosis, and that it originates in the vital properties of the intestinal epithelium, Prof. Heidenhain argues, is rendered probable by the fact that, on exposing the intestine to the action of sodium fluoride—a poison which destroys the vitality of the intestinal epithelium—salt solutions are absorbed by osmosis, and by osmosis alone; and therefore none of the water is taken up from hypertonic or isotonic solutions, and none of the salt from hypotonic solutions. By means of these control experiments, Prof. Heidenhain is justified in concluding that the forces, by which absorption of salt solutions from the intestine is brought about, are twofold: one depending upon differences of osmotic pressure, the other upon the life of the epithelium. The work which this latter 'physiological' force is capable of doing can be measured by balancing it against osmotic pressure.

It was evidently possible to unravel the problem before us on precisely these lines. The details of the experiments that we have carried out will show how closely we have followed Prof. Heidenhain's method.

*Method.* We have experimented on dogs weighing from 4 to 8 kilos. Morphia was injected an hour or two beforehand. A. C. E. mixture was used to maintain anæsthesia. From 60 to 100 c.c. of different solutions, warmed to the body temperature, were introduced into the pleural cavity by means of an exploring needle attached to a burette. At the end of periods varying from half-an-hour to two hours, blood was drawn off from the femoral artery. The dog was then killed, the chest opened and the fluid removed by means of a pipette. We found that, in this way, practically the whole amount of fluid present in the chest can be removed, the loss being less than 1 c.c. The osmotic pressures of the fluid put in, of the fluid recovered, and of the blood-serum separated by the centrifugal machine, were determined by taking the freezing points with Beckmann's apparatus. The solutions used by us were mostly solutions of sodium chloride—in a few cases, of sodium sulphate and of magnesium sulphate, and in others, sodium fluoride was added to sodium chloride.

The solutions were prepared by weighing out recrystallized dried salts, but were also estimated by the methods employed for estimating the salts in the fluids recovered from the chest at the end of the experiment. In estimating sodium chloride, we employed Volhardt's method: 20 c.c. of the fluid were dried in the oven, after the addition of some bicarbonate of soda; the solid residue was heated to a dull red heat to char the organic matter present, and then extracted with nitric acid, and the extract titrated with silver nitrate and sulphocyanide of potassium. The chlorides in the serum were estimated in the same way. Sodium sulphate was estimated gravimetrically as barium sulphate; magnesium sulphate as magnesium pyrophosphate, by precipitation with sodium phosphate and igniting.

Proceeding to the examination of our results, we may at the outset clear the ground by saying that we find no evidence of any such marked cell-activity in the case of the pleural cavity as Prof. Heidenhain finds in the intestine.

*Injection of hypertonic solutions.* (*v.* Table I.) Whereas in the intestine a 2 °/₀ NaCl solution is absorbed, we have in nine experiments with hypertonic solutions only once found any absorption to take place,

TABLE I.

*Hypertonic Solutions of Sodium Chloride.*

| | | | Injected | | | Recovered | | | Absorbed | | Serum | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of Exp. | Weight of Dog | Duration of Exp. | Volume in c.c. | °/₀ NaCl | Δ of fluid | Volume in c.c. | °/₀ NaCl | Δ of fluid | Volume in c.c. | Grammes NaCl | Δ | °/₀ NaCl |
| 1 | | 30′ | 60 | 1·2 | ·72 | 61 | ·93 | | − 1 | ·163 | | |
| 2 | | 30′ | 80 | " | " | 83 | ·94 | | − 3 | ·18 | | |
| 3 | | 2° | 60 | " | " | 57 | ·8 | | + 3 | ·264 | | |
| 19 | | 30′ | 80 | 1·22 | ·74 | 85 | 1·03 | ·66 | − 5 | ·10 | ·60 | ·68 |
| 20 | | 30′ | 60 | 1·5 | ·91 | 64 | 1·21 | ·785 | − 4 | ·13 | ·61 | ·72 |
| 4 | | 30′ | 80 | " | " | 97 | 1·02 | | − 17 | ·21 | | ·63 |
| 25 | | 2° | 80 | " | " | 95 | ·88 | ·61 | − 15 | ·37 | ·595 | ·74 |
| 5 | | 2° | 80 | " | " | 94 | ·815 | | − 14 | ·44 | | ·69 |
| 10 | | 2° | 80 | " | " | 90 | ·84 | | − 10 | ·44 | | ·71 |

and that was when we had injected a comparatively weak solution, 1·2 °/₀ NaCl, and had left it in the pleural cavity for a comparatively long time, 2 hours, and even in this case only 3 c.c. were absorbed. In every other instance the volume of fluid recovered was greater than that of the fluid injected. The action of osmosis is practically unopposed.

*Injection of hypotonic solutions.* (*v.* Table II.) So, too, is it in the experiments with hypotonic solutions; in accordance with simple osmosis the volume of fluid in every case is diminished. One striking fact will be observed in the figures given in Table II., that the rate of absorption in the half-hour experiments is very rapid compared with that in the two-hour experiments. In the former, the average amount

TABLE II.

*Hypotonic Solutions of Sodium Chloride.* ·5 °/₀. $\Delta = \cdot 34$.

| | | | Injected | | Recovered | | | | Absorbed | | | | Serum | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of Experiment | Weight of Dog | Duration of Exp. | Volume injected in c.c. | Weight of NaCl injected in grammes | Volume in c.c. | °/₀ NaCl | Weight NaCl in grs. | Δ of fluid recorded | Volume in c.c. | Grammes NaCl | Fraction of volume injected | Fraction of weight of salt injected | Δ | °/₀ NaCl |
| 6 | | 30′ | 80 | ·4 | 44 | ·72 | ·316 | | 36 | ·084 | ·44 | | ·615 | ·63 |
| 7 | | ,, | 50 | ·25 | 34 | ·69 | ·234 | | 16 | ·016 | ·2 | | ·61 | ·63 |
| 16 | | ,, | 80 | ·4 | 48 | ·71 | ·339 | ·53 | 32 | ·061 | ·4 | | ·57 | ·66 |
| 18 | | ,, | 80 | ·4 | 56 | ·635 | ·355 | ·57 | 24 | ·045 | ·275 | | ·64 | |
| 8 | | 2° | 80 | ·4 | 40 | ·75 | ·3 | | 40 | ·1 | ·5 | | ·575 | ·65 |
| 9 | | ,, | 60 | ·3 | 28 | ·69 | ·193 | | 32 | ·107 | ·53 | | ·565 | ·61 |
| 21 | | ,, | 80 | ·4 | 44 | ·71 | ·31 | ·60 | 36 | ·09 | ·45 | | ·62 | ·67 |

absorbed is ·39 of the total amount injected, in the latter, it is only ·49; so that the extra 1½ hours has led to the absorption of only an additional ·1 of the original amount of fluid, not more, that is to say, than 8 c.c., which corresponds to a rate of 5 c.c. an hour. An explanation of this is given by the fact that, by the end of half-an-hour, osmotic equilibrium has been almost established (in Nos. 16 and 18 about four-fifths of the difference between the osmotic pressures of the injected solution of the serum have been cancelled). Therefore we may suppose that, in the two-hour experiments after the first half-hour, the same conditions had been brought about and so osmotic absorption had really come to an end. From these experiments it is clear therefore that absorption is rapid, so long as it is determined by osmosis, and after that, the rate sinks to about 5 c.c. an hour.

*Injection of isotonic solutions.* (*v.* Table III.) Now turning to Table III, in which are given the results of experiments with an isotonic fluid, we see that absorption in this case also takes place, but that it is slow, that the rate is the same in the half-hour as in the two-hour experiments, and that the rate is the same as we found for ·5 % solutions after the establishment of osmotic equilibrium. This absorption of isotonic fluid, which occurs at a fairly uniform rate therefore in all our experiments, is the first possible indication of any cell-activity that we have come across.

TABLE III.

*Isotonic Solutions.* 1 % $NaCl = \Delta =$ ·61.

| | | | Injected | | Recovered | | | | Absorbed | | | | Serum | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of Experiment | Weight of Dog | Duration of Exp. | Volume in c.c. | Grammes NaCl | Volume in c.c. | % NaCl | Grammes NaCl | Δ | Volume in c.c. | Grammes NaCl | Fraction of volume injected | Fraction of salt injected | Δ | % NaCl |
| 12 | | 30′ | 100 | 1·0 | 96 | ·963 | ·924 | | 4 | ·076 | | | | ·61 |
| 17 | | 30′ | 80 | ·8 | 78 | ·915 | ·714 | ·595 | 2 | ·086 | | | ·595 | ·7 |
| 14 | | 2° | 80 | ·8 | 70 | ·8 | ·56 | | 10 | ·24 | | | | ·60 |

But even in this case it is at least possible that the absorption may be effected by the lymphatics, by some such mechanism as that described by Dybkowsky[1]. In an experiment not included in Table

[1] Ludwig's *Arbeiten*, 1866, p. 191. The conclusions here arrived at are that, with each inspiration, the intercostal subpleural lymphatic network is opened out and filled with fluid from the pleural cavity; the forces effecting this being the elasticity of the lung on the one side and the tightening of the intercostal fascia by the inspiratory movements on the other. With each expiration the intercostal fascia is relaxed and bulges inwards, the capacity of the subpleural lymphatic network is diminished and its contents are emptied into the efferent lymphatic vessels. This pumping action varies with the vigour of the inspiratory movements and the elastic force exerted by the lung. There is no evidence of absorption by the visceral or mediastinal pleural, and the subpleural lymphatic network is wanting over the ribs.

III, we found that 100 c.c. of 1 % NaCl solution had entirely disappeared from the pleural cavity after 20 hours. Absorption at the rate of 5 c.c. an hour would be sufficient to account for this. We repeated this experiment, after ligaturing the thoracic duct and the lymphatic duct on the right side of the neck, and found that the fluid had disappeared in this case also. Even here however the possibility of its having been taken by the lymphatics is not excluded. It is true that, if the thoracic duct be ligatured at the same time as the bile-duct, no staining of the tissues with bile takes place. But this only proves that the lymphatic vessels of moderate calibre, such as those leading from the liver, do not allow filtration of the bile out into the tissues. But the lymph capillaries, which they drain, are a distinct and isolated system shut up in the liver and almost completely invested by the capsule of the liver, whereas the subpleural lymphatic capillaries form a network continuous with those of the mediastinum, the neck, and the subperitoneal connective tissue; and there is nothing to prevent fluid, which is sucked up out of the pleural cavity into the subpleural lymphatic network, from distributing itself far and wide, when the ordinary outlet by the lymphatic ducts is closed, much as emphysema spreads in the subcutaneous tissue all over the body. Moreover, in the animal, whose lymphatic ducts we tied, there was visible infiltration of the posterior mediastinum, especially at the lower part and between the pillars of the diaphragm. In a control experiment, in which both thoracic ducts were tied, without the injection of

TABLE IV.

*Effect of Sodium Fluoride.*

| | | | Injected | | | | Recovered | | | Absorbed | | Serum | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of Experiment | Weight of Dog | Duration of Exp. | Volume in c.c. | % NaCl | % NaFl | $\Delta$ | Volume in c.c. | % NaCl | $\Delta$ | Volume in c.c. | Grammes NaCl | $\Delta$ | % NaCl |
| 13 | | 2° | 60 | ·952 | ·095 | ·68 | 50 | ·727 | | 10 | ·21 | ·61 | ·59 |
| 22 | | 2° | 80 | ·39 | ·075 | ·315 | 40 | | ·61 | 40 | | ·585 | |
| 24 | | 2° | 80 | ·2 | ·2 | ·33 | 42 | ·66 | ·61 | 38 | – ·11 | ·60 | ·645 |
| 11 | | 30′ | 80 | ·5 | ·1 | ·395 | 60 | ·63 | ·605 | 20 | ·022 | ·60 | ·60 |

any fluid into the pleura, and the animal killed the following day, no such infiltration of mediastinal tissues was observed.

On the supposition however that the cells of the pleura and its capillaries had something to do with this slow removal of isotonic solutions, we tried the effect of adding to our solutions sodium fluoride, upon which Heidenhain relied for control proof of the work done by the epithelium. In Table IV. we give the results of experiments in which we made use of this salt.

In No. 13 the fluid we injected was slightly hypertonic ($\Delta = \cdot 68$), but so far from there being any sign of arrest of an active absorption, the same amount was absorbed as we have shown is usual with isotonic solutions, viz., 5 c.c. an hour. In No. 11, the addition of ·1 % NaCl to the ·5 % solution of sodium chloride lowers its freezing point from $- \cdot 34^\circ$ to $- \cdot 395^\circ$. In this case, of the 80 c.c. fluid injected, 60 c.c. were recovered at the end of half-an-hour. If the sodium fluoride had not been added we should have expected to recover 50 c.c. It is evident therefore that the retarding effect of the sodium fluoride on the absorption is due to the fact that the addition of this salt caused a rise of total osmotic pressure of the fluid injected. In Nos. 22 and 24, we tried to arrange the proportion of the two salts so as to make the osmotic pressure correspond to that of ·5 % NaCl solution. We were most successful in No. 24 ($\Delta$ of the solution used $= \cdot 33$, $\Delta$ of ·5 % NaCl $= \cdot 34$), and the amount of fluid absorbed was 38 c.c., the mean between 40 and 36 c.c., which were the amounts absorbed in the same time from the same volume of ·5 % NaCl solution (cp. Nos. 8 and 21 in Table II.). In No. 22, rather more fluid was absorbed (40 c.c.), but this only corresponds to a rather lower osmotic pressure. ($\Delta = \cdot 315$.)

The correspondence here is so close that we can but infer either that the cells do not take any part at all in the absorption of the fluid, or that, if they do, sodium fluoride has no effect upon them. If the latter is the case, it is remarkable, because we used in No. 24 five times the strength of sodium fluoride solution that Prof. Heidenhain found sufficient for establishing his control, and the pleura was covered with ecchymoses.

Although therefore we have not succeeded in proving that the slow absorption, which is not accounted for by osmosis, is effected by the lymphatics, these experiments with sodium fluoride show that the absorption cannot be ascribed to active intervention of the cells of the pleural endothelium, and that we must therefore look rather for some mechanical explanation.

So far we have confined our attention to the disappearance of the water. If we consider now the conditions determining the absorption of the dissolved salt, we find nothing in the experiments recorded in Tables I. and III., which would point to an active absorption, since in these the amount of salt absorbed seems to be determined entirely by the partial pressure of the salt in the fluid relatively to that in the serum. In Table II., however, experiments are given in which salt was absorbed from a solution which contained less of this salt than did the blood-plasma. How is this absorption to be explained?

*The Significance of the Absorption of Salt from hypotonic Solutions.*

Great stress is laid both by Heidenhain and by his pupil Orlow, on the fact that, in the peritoneal cavity as well as from the intestine, salt may be taken up from fluids containing a smaller percentage of this substance than does blood-plasma, and they regard this absorption as pointing indubitably to an active intervention of living cells in the process. This argument requires examination.

The physical conditions in our experiments are these: ·5 % NaCl solution ($\Delta = -·34$) is separated by living membranes from blood-plasma containing from ·61 to ·67 % NaCl, and, in addition to this, other soluble matter, giving a total osmotic pressure equivalent to that of a 1 % salt solution ($\Delta = -·61$). We have seen how these osmotic conditions determine a rapid passage of water from the pleural cavity into the blood. If the membrane separating the circulating blood from the pleural cavities were perfectly 'semi-permeable,' i.e., permeable to water, but impermeable to dissolved salts, it is evident that the passage of water through the membrane would be regulated solely by the differences between the total osmotic pressures on each side. Water would pass from the pleural fluid into the blood, until the osmotic pressure of the remaining pleural fluid was exactly equal to that of the blood, i.e. 1 % NaCl. But, since the membrane is impermeable to salts, this equalisation of osmotic pressures must be effected simply and solely by the passage of water, so that, at the end of the experiment, the pleural fluid would contain 1 % sodium chloride while the surrounding blood-plasma would still possess its normal amount—·6 to ·7 % NaCl, together with other salts bringing its osmotic pressure up to that of 1 % NaCl solution.

If, on the other hand, the membrane were permeable to salt as well as to water, the course of events would be as follows. At first water would pass out of the pleura, and salt would diffuse in, until the percentage of NaCl in the fluid was equal to that in the blood-plasma.

There would now be an equal partial pressure of NaCl on the two sides of the pleural membrane, but the total osmotic pressure of the blood-plasma would still be higher than that of the pleural fluid. Water would still continue to be absorbed by the blood-vessels. As soon, however, as more water passed out from the pleural cavity, the percentage of salt in the fluid left would be raised above that in the blood, and salt would immediately pass over into the vessels, and this would go on until time had been given for enough of the other soluble matter affecting the osmotic pressure of the blood to pass from the vessels into the pleural sac. Osmotic equilibrium would be then established: salt, as well as water would have been absorbed, and at no time would the percentage of sodium chloride in the pleural fluid have been perceptibly higher than that in the blood-plasma.

This absorption of salt from hypotonic solutions is well illustrated by the following experiment: 125 c.c. of ·5 % NaCl solution were put in a parchment paper dialyser and suspended in a fluid containing ·5 % NaCl with 10 % $KNO_3$. The next morning the fluid in the dialyser was found to have diminished to 75 c.c. The percentage of salt however in the remaining fluid was the same as at the beginning of the experiment, i.e. ·5 %, showing that salt, as well as water, had passed from the dialyser into the outer fluid.

It is evident then that neither the raising of the percentage of a salt in any fluid above that of the same salt in the plasma, nor the passage of a salt from a hypotonic fluid into the blood-plasma, can afford in itself any proof of an active intervention of cells in the process.

In the case of the pleura, we seem to have a membrane which is very imperfectly semipermeable. It is permeable to salts, but presents rather more resistance to their passage than to the passage of water. Hence, on injecting ·5 % NaCl solution into the pleural cavity, water passes from the pleural fluid into the blood, until the percentage of sodium chloride in the fluid is raised perceptibly above that in the blood-plasma. The limit of the resistance of the pleural membrane to the passage of salt is however soon reached, and then salt passes from pleural fluid into blood; but in every case this passage is from a region of higher to a region of lower partial pressure. Hence at the end of the experiment, we find a higher percentage of salt in the pleura than in the blood vessels, although the total amount of salt in the pleural fluid is less than that originally put in, or, in other words, salt has been absorbed.

That this course of reasoning is correct is shown by the results given in Table II. and also those of two experiments given in Table V. (Nos. 23 and 26). In these two experiments the percentage of salt in the fluid introduced was so arranged as to be already slightly higher than that in the serum. At the end of the experiments, the fluid, which originally contained ·75 % NaCl, as against ·68 and ·73 % NaCl in the serum, was found, after half-an-hour to contain ·82 and ·85 % NaCl; water had been absorbed and some of the salt.

TABLE V.

*·75 % solutions of Sodium Chloride at different Temperatures.*

| No. of Experiment | Weight of Dog | Duration of Exp. | Injected: Volume in c.c. | Injected: Temperature | Injected: % NaCl | Injected: Δ of fluid | Recovered: Volume in c.c. | Recovered: % NaCl | Recovered: Δ of fluid | Absorbed: Volume in c.c. | Absorbed: Grammes NaCl | Serum: Δ | Serum: % NaCl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 7·7 | 30′ | 80 | 40° | ·75 | ·47 | 65 | ·82 | ·575 | 15 | ·07 | ·61 | ·685 |
| 26 | 5 | 30′ | 80 | 40° | ·75 | ·47 | 50 | ·85 | ·565 | 30 | ·175 | ·605 | ·73 |
| 34 | 6 | 30′ | 80 | 75° | ·75 | ·47 | 64 | ·86 | | 16 | ·05 | | ·67 |
| 35 | 7·5 | 5° | 80 | 88° | ·75 | ·47 | 38 | ·715 | ·62 | 42 | ·37 | ·61 | ·69 |

Our attempts to alter the permeability of the pleural wall have given only negative results. Thus in Nos. 34 and 35 (Table V.) we tried to alter the conditions by scalding the pleura and introduced the fluid at a temperature of about 80° C.; and although, on opening the chest, we found that the membrane looked opaque and otherwise damaged, there was no evidence that we had altered the course of the absorption in any way. In Exp. No. 34, the amount of fluid introduced and the duration of the experiment were the same as in the Exps. 23 and 26, in which the fluid was introduced at the body-temperature. It will be seen however that the results are practically identical.

Further attempts to alter the permeability of the pleural membrane by the addition of sodium fluoride to the solutions injected into the pleura gave only negative results.

But the fact that, by these means, we were unable to modify our results, only gives support to the view we have taken of the absorption of salt from hypotonic solutions. If we had here the manifestation of a vital function of the pleural endothelium, the fact, that these cells behave in precisely the same way after being scalded or poisoned with sodium fluoride as under normal conditions, would be very difficult to explain.

In Table VI. are given the results of a few experiments made with solutions of other salts. These agree in all essentials with the experiments in which sodium chloride solutions were used. Although for the sake of simplicity, we have not made use of them in the argument in this paper, they may be here quoted to show that the same laws determine the absorption of other salts as of sodium chloride.

TABLE VI.

*Solutions of Sodium Sulphate and Magnesium Sulphate.*

| No. of Experiment | Weight of Dog | Duration of Exp. | Injected: Volume in c.c. | Injected: % $Na_2SO_4$ | Injected: Δ | Recovered: Volume in c.c. | Recovered: % $Na_2SO_4$ | Recovered: Δ | Absorbed: Volume in c.c. | Absorbed: $Na_2SO_4$ in grms. | Serum: Δ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 28 | | 1° | 60 | 2·16 | ·665 | 70 | 1·32 | ·625 | – 10 | ·371 | ·60 |
| 31 | | 1° | 60 | 2·16 | ·665 | 68 | 1·47 | ·635 | – 8 | ·30 | ·62 |
| 29 | | 30′ | 80 | 1·06 | ·37 | 60 | 1·16 | ·53 | 20 | ·152 | ·60 |
| 33 | | 30′ | 80 | ·906 | ·30 | 56 | ·909 | ·525 | 24 | ·216 | |
| | | | | % $MgSO_4$ | | | % $MgSO_4$ | | | $MgSO_4$ in grammes | |
| 32 | | 30′ | 80 | 1·036 | ·21 | 59 | 1·069 | ·495 | 21 | ·198 | ·59 |